

Review

Functional Selectivity and Antidepressant Activity of Serotonin 1A Receptor Ligands

Zdzisław Chilmonczyk ^{1,2,*}, Andrzej Jacek Bojarski ³, Andrzej Pilc ³ and Ingebrigt Sylte ⁴

¹ National Medicines Institute, Chełmska 30/34, 00-725 Warszawa, Poland

² Institute of Nursing and Health Sciences, University of Rzeszów, W. Kopisto 2A, 35-310 Rzeszów, Poland

³ Institute of Pharmacology, Polish Academy of Sciences, Smetna 12, 31-343 Kraków, Poland; E-Mails: bojarski@if-pan.krakow.pl (A.J.B.); nfpilc@cyf-kr.edu.pl (A.P.)

⁴ Faculty of Health Sciences, University of Tromsø—The Arctic University of Norway, No-9037 Tromsø, Norway; E-Mail: ingebrigt.sylte@uit.no

* Author to whom correspondence should be addressed; E-Mail: z.chilmonczyk@nil.gov.pl; Tel.: +48-22-851-4369 (ext. 448); Fax: +48-22-840-6330.

Academic Editor: Kenji Hashimoto

Received: 2 July 2015/ Accepted: 31 July 2015/ Published: 7 August 2015

Abstract: Serotonin (5-HT) is a monoamine neurotransmitter that plays an important role in physiological functions. 5-HT has been implicated in sleep, feeding, sexual behavior, temperature regulation, pain, and cognition as well as in pathological states including disorders connected to mood, anxiety, psychosis and pain. 5-HT_{1A} receptors has for a long time been considered as an interesting target for the action of antidepressant drugs. It was postulated that postsynaptic 5-HT_{1A} agonists could form a new class of antidepressant drugs, and mixed 5-HT_{1A} receptor ligands/serotonin transporter (SERT) inhibitors seem to possess an interesting pharmacological profile. It should, however, be noted that 5-HT_{1A} receptors can activate several different biochemical pathways and signal through both G protein-dependent and G protein-independent pathways. The variables that affect the multiplicity of 5-HT_{1A} receptor signaling pathways would thus result from the summation of effects specific to the host cell milieu. Moreover, receptor trafficking appears different at pre- and postsynaptic sites. It should also be noted that the 5-HT_{1A} receptor cooperates with other signal transduction systems (like the 5-HT_{1B} or 5-HT_{2A/2B/2C} receptors, the GABAergic and the glutaminergic systems), which also contribute to its antidepressant and/or anxiolytic

activity. Thus identifying brain specific molecular targets for 5-HT_{1A} receptor ligands may result in a better targeting, raising a hope for more effective medicines for various pathologies.

Keywords: serotonin 1A receptors; receptor trafficking; antidepressant activity

1. Introduction

Serotonin is a monoamine neurotransmitter that plays an important role in physiological functions, such as sleep, feeding, sexual behavior, temperature regulation, pain, and cognition, as well as in pathological states including mood disorders, anxiety disorders, psychosis, and pain disorders. Medications that increase the level of 5-HT, such as the selective serotonin reuptake inhibitors, are used as treatments of depression and anxiety. The seven 5-HT receptor classes consist of 5-HT₁, 5-HT₂, 5-HT₃, 5-HT₄, 5-HT₅, 5-HT₆, 5-HT₇, which are further subdivided into 14 receptor subclasses. All of these receptors—except for 5-HT₃ receptor class, which belongs to the family of ligand-gated ionic channels and is permeable to Na⁺, K⁺, Ca²⁺ (and other cations)—belong to the superfamily of seven-transmembrane-domain, G protein-coupled receptors (GPCRs). For serotonin GPCRs, three main types of primary coupling to G proteins have been described. The 5-HT_{1A} receptors activate G_i/G_o proteins, the 5-HT_{2A} receptors activate G_q/G₁₁, and the 5-HT₄, 5-HT₆, and 5-HT₇ activate G_s [1].

5-HT neurons show a number of anatomical and physiological characteristics, some of which are shared by noradrenergic neurons [2–4]:

- Low number of neurons: 250,000 5-HT neurons in the human brain (out of a total of 10¹¹).
- Innervation of the whole neuraxis with extensive axon branching (4106 nerve terminals/mm³ in neocortex).
- Slow and regular discharge (pace maker neurons): strong homeostasis.
- Neuronal activity dependent on sleep-wake cycles (REM-off neurons).
- Very sensitive to self-inhibition through activation of 5-HT_{1A} autoreceptors.
- Rich neurochemistry: 14 different postsynaptic receptors.
- Implication in a large number of physiological functions.
- Mutual control with monoaminergic cell groups.

5-HT neuronal activity is tightly controlled by several afferent pathways, including glutamatergic inputs from the forebrain (e.g., prefrontal cortex, PFC), monoaminergic, peptidergic and local GABAergic inputs [5]. Overall, this means that serotonergic activity and 5-HT release in the forebrain is very tightly controlled by a number of pre- and postsynaptic mechanisms, which have been selected through evolution to keep a tonic and regular activity of 5-HT neurons. As a general rule, antidepressant drugs aim at enhancing serotonergic activity, therefore interfering with the homeostasis of the 5-HT system and activating self-adaptive mechanisms that limit the full antidepressant action [2].

2. 5-HT_{1A} Receptor Distribution

While all 5-HT receptor subtypes are localized post-synaptically on 5-HT target cells, the 5-HT_{1A}, 5-HT_{1B/D}, and 5-HT_{2B} receptors are also localized on 5-HT neurons [6]. 5-HT_{1A} receptors are present in high densities in limbic brain areas (hippocampus, lateral septum), cortical areas (particularly prefrontal and entorhinal cortex), as well as in the raphe nuclei, both dorsal and median [7]. They are being found in the soma, in dendrite, in some cases in the hillock of neurons, and in the cell body and processes of astrocytes [8]. Stimulation of 5-HT_{1A} receptors in prefrontal cortex enhances forebrain catecholamine release, an effect possibly involved in the antidepressant action of the receptor agonists. The physiological release of 5-HT inhibits the activity of PFC pyramidal neurons due to the direct stimulation of 5-HT_{1A} receptors located on the neurons (for a review see [9]).

Presynaptically, the 5-HT_{1A} receptor is the major somatodendritic autoreceptor on the soma and dendrites of serotonergic neurons, where it acts as a “brake” to inhibit the activity of the entire 5-HT system and is thought to delay the antidepressant response [10]. Local release of 5-HT in the raphe nuclei from axonal collaterals or crosstalk between different 5-HT neurons may diminish neuronal firing and produce a negative feedback regulation of transmitter release and may add an extra level of topographical specification. Consistent with their role in regulating serotonergic tone, activation of autoreceptors limits the initial increase of the extracellular 5-HT levels induced by selective serotonin reuptake inhibitors (SSRIs), and delay their therapeutic response [11,12].

Richardson-Jones *et al.* obtained transgenic mice with normal (referred as 1A-high) and low 5-HT_{1A} autoreceptor levels. It was shown that compared to 1A-high mice, 1A-low mice have indistinguishable levels of 5-HT_{1A} heteroreceptor expression, but displayed about 30% less autoreceptor expression than did the 1A-high mice. In that model, when the serotonergic system was activated, higher intrinsic 5-HT_{1A} autoreceptor levels resulted in lower raphe firing rate (Figure 1). The obtained data also suggested that, at baseline (*i.e.*, non-stressful conditions), levels of serotonin did not differ between the 1A-high and 1A-low mice [13,14].

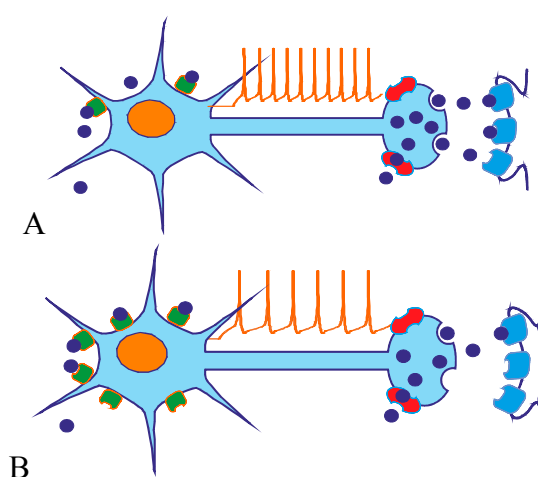


Figure 1. Raphe neurons with low (A) and high (B) 5-HT_{1A} receptor density. Mice with high somatodendritic 5-HT_{1A} expression have lower basal firing rate (and elevated behavioral despair level). Blue dots—serotonin, red shape—SERT, green shape—5-HT_{1A} receptor (according to [13,14]).

Postsynaptic 5-HT_{1A} heteroreceptors are expressed in target areas receiving serotonergic innervation. They are located on non-serotonergic neurons, primarily in the limbic areas, such as on the dendrites and soma of glutamatergic pyramidal neurons, and axon terminals of GABAergic and cholinergic neurons. The heteroreceptors are particularly enriched in the hippocampus, where immunohistochemistry and radioligand binding have demonstrated high receptor levels in the stratum radiatum of CA1 and the granule cell layer of the dentate gyrus, and moderate levels in CA3. 5-HT_{1A} heteroreceptors are also highly expressed in the entorhinal cortex, frontal cortex, and lateral septum, and moderately expressed in the amygdala, superior colliculus, piriform cortex, and interpeduncular nucleus, as well as in several hypothalamic and thalamic nuclei. Activation of 5-HT_{1A} heteroreceptors on these distinct neurons mediates hyperpolarizing response to released serotonin and usually reduces neuronal excitability and firing [10,14].

Aside from 5-HT_{1A}, the 5-HT_{1B} receptor is also thought to serve as an autoreceptor. 5-HT_{1B} receptors are expressed in the central nervous system (CNS), concentrated in the basal ganglia, striatum, and frontal cortex. In addition, the receptor may also act as a terminal heteroreceptor controlling the release of other neurotransmitters, such as acetylcholine, glutamate, dopamine, noradrenaline, and γ -aminobutyric acid [15,16]. The receptor is also found on cerebral arteries and other vascular tissues. The putative 5HT_{1B} receptor agonist, anpirtoline, has analgesic and antidepressant-like properties in rodents, and 5-HT_{1B} receptor KO mice were reported to be both highly aggressive and have an increased preference for alcohol [16,17].

Studies investigating the relationship between 5-HT_{1A} and 5-HT_{1B} receptors showed that 5-HT_{1A/1B} knockouts had increased extracellular serotonin in the hippocampus, suggesting that the pairing of SSRI with 5-HT_{1A/1B} antagonist might prove to be a potent treatment for anxiety and depression [18]. 5-HT_{1B} receptor agonist (CP 94253) and antagonist (SB 216641) have been shown to be effective in preclinical models of anxiety and CP 94253 was also effective in the model of depression (forced swim test—FST) [19]. It was also shown that the activation of 5-HT_{1B} heteroreceptors induces antidepressant-like effect in mice [20].

3. 5-HT_{1A} Receptor Regulated Transcription Pathways

3.1. Adenylate Cyclase

The primary coupling linkage of the 5-HT_{1A} receptor (and of all 5-HT₁ receptors) is to the inhibition of adenylate cyclase (AC) and decrease protein kinase A (PKA) activity. However, the 5-HT_{1A} receptor couples to the broadest panel of second messengers of any of the 5-HT receptors [21]. This receptor has been reported to activate or inhibit various enzymes, channels, and kinases, and to stimulate or inhibit production of diverse soluble second messengers. The receptor has been found to inhibit and activate AC and phospholipase C (PLC), to stimulate nitric oxide synthase (NOS) and a nicotinamide adenine dinucleotide phosphate (NADP) oxidase-like enzyme, to activate K⁺ channels and high conductance anion channels, to inhibit Ca²⁺ conductance and inhibit or stimulate Ca²⁺ mobilization, and regulate a number of channels and transporters (Figure 2) [22–24]. The 5-HT_{1A} receptor can activate protein kinase C (PKC), Src kinase, and mitogen-activated protein kinases (MAPKs), activate or inhibit phosphatidyl

inositol hydrolysis, and stimulate production of reactive oxygen species (both H_2O_2 and superoxide) and arachidonic acid [1].

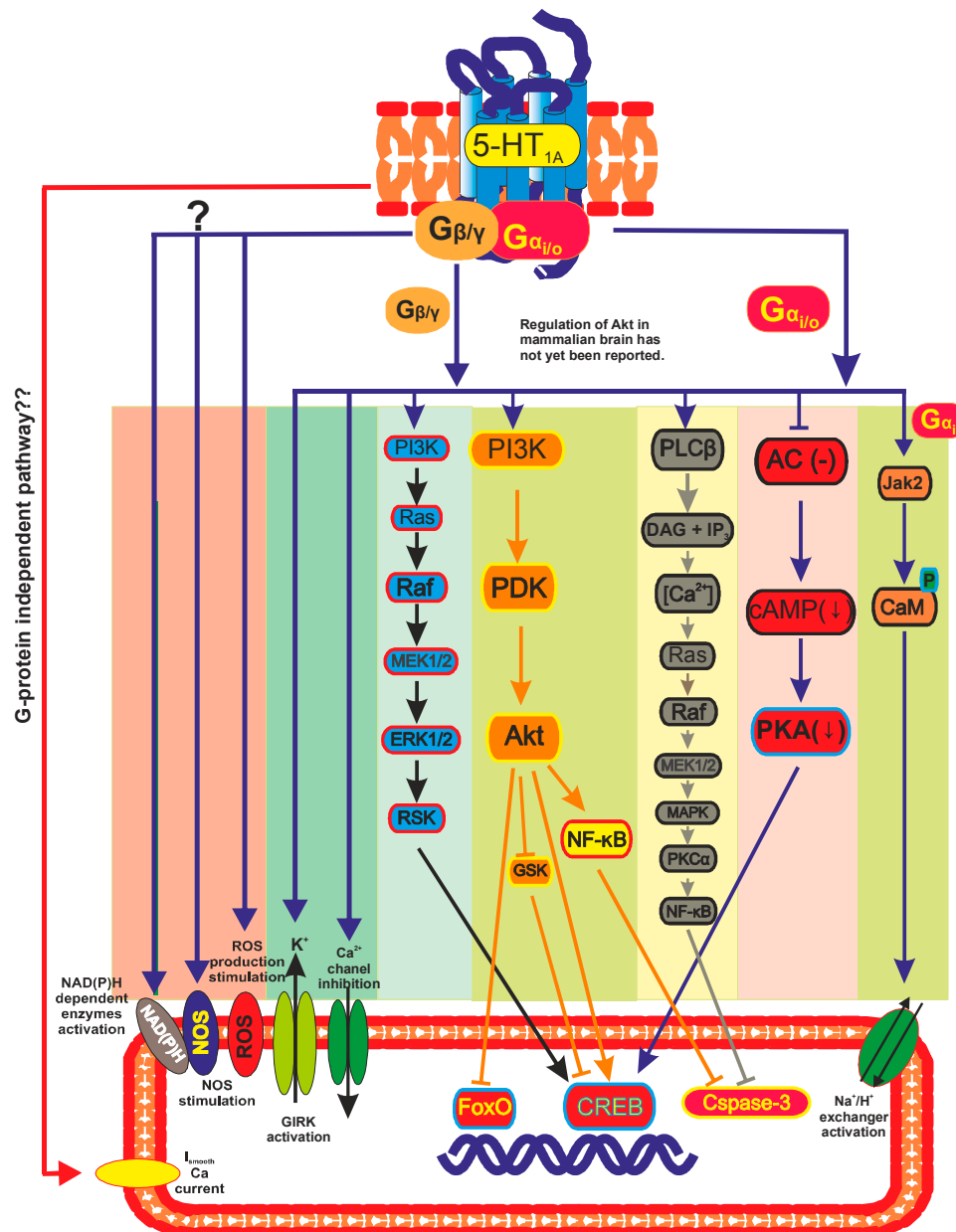


Figure 2. Signal transduction pathways of the 5-HT_{1A} receptor [22–24].

Despite the diversity of second messengers activated, all of the signals are almost completely sensitive to *pertusis* toxin, implicating G_{i/o} proteins in the signals initiated by the 5-HT_{1A} receptor [21]. 5-HT_{1A} heteroreceptors were shown to couple to G_{ai}-induced inhibition of adenylate cyclase [7,25] while for 5-HT_{1A} autoreceptors the situation is not so clear. Clarke *et al.* [25] found that 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and dipropyl-5-carboxamidotryptamine (5-HT_{1A} receptor agonists) did not inhibit forskolin-stimulated adenylate cyclase activity in raphe region homogenates. On that basis it was accepted that the autoreceptor located in the dorsal raphe nucleus (DRN) does not exhibit coupling to AC-. It was found, however, that also in the raphe nucleus the 5-HT_{1A} autoreceptor may exhibit coupling to G_{ai3} and negatively regulate AC. Buspirone, a partial agonist of 5-HT_{1A} receptor,

reduced the presynaptic adenylate cyclase activity in raphe nuclei, the effect being reversed by WAY 100,635, the 5-HT_{1A} receptor antagonist. Such behavior was not, however, observed for flibanserin and 8-OH-DPAT (and other 5-HT_{1A} receptor agonists), which did not exhibit coupling to AC activity in raphe nuclei [26,27]. Thus, those ligands exhibited regional ligand-directed receptor trafficking.

Constitutive activity of 5-HT_{1A} receptors has not been extensively examined. Constitutive activity of G-protein (G α) has been demonstrated in human 5-HT_{1A} receptors stably expressed in transfected cell lines, as well as in the native rat 5-HT_{1A} receptors in hippocampal membranes, as revealed by the inhibitory effect of inverse agonists, such as spiperone or methiothepin, on basal guanosine-5'-O-(3-[³⁵S]thio)-triphosphate ([³⁵S]GTP γ S) binding. In the native rat 5-HT_{1A} receptors, using anti-G α -antibody, it was observed that spiperone and methiothepin reduced basal [³⁵S] GTP γ S binding to G α in a concentration-dependent manner to 90% of basal. The inhibition of basal [³⁵S] GTP γ S binding induced by spiperone and methiothepin was antagonized by WAY 100,635 (5-HT_{1A} selective neutral antagonist) in a concentration-dependent manner, thus indicating that this inverse agonism was mediated by 5-HT_{1A} receptors [28]. In the passive avoidance paradigm, the 8-OH-DPAT-induced decrease in PKA activity in the hippocampus caused increased protein phosphatase-1 activity and a reduction of training-induced phosphorylation of calcium/calmodulin-dependent protein kinase II (CaMKII), and this signaling effect was accompanied by cognitive deficits [29]. Therefore, inhibition of adenylate cyclase and PKA activity may mediate 5-HT_{1A} receptor-regulated behaviors.

3.2. GIRK and Calcium Channel

In neurons, activation of the 5-HT_{1A} receptor activates G protein-coupled inwardly-rectifying potassium channels (GIRKs) [30] in the hippocampus [31–33] and in the DRN [25,34], an action that profoundly hyperpolarizes neurons and decreases firing [35–38]. The activation of GIRKs is primarily mediated by G protein $\beta\gamma$ subunits upon receptor activation [39–41]. The ability of 5-HT_{1A} receptors to activate GIRK-induced hyperpolarizing currents allows them to have a strong effect on neuronal firing and excitability [34], a physiological process that may be linked to 5-HT_{1A} receptor-regulated behaviors [42].

5-HT_{1A} receptor activation also inhibits voltage-gated calcium channel activity to reduce calcium entry [43–45]. 5-HT_{1A} receptor-mediated inhibition of Ca²⁺ currents in dorsal raphe was found to be inhibited by a peptide inhibitor of G protein $\beta\gamma$ subunit [46].

3.3. ERK/MAPK Pathway

Another major pathway of the 5-HT_{1A} receptor is by activation of extracellular signal-regulated protein kinase (ERK) (or MAPK), which has been implicated in various aspects of cell proliferation and differentiation [47]. 5-HT_{1A} receptors were first reported to activate ERK by phosphorylation in non-neuronal cells expressing 5-HT_{1A} receptors [48,49]. This effect can be stimulated in transfected human 5-HT_{1A} receptors via a pathway that shares many of the mediators of growth signals initiated by receptor tyrosine kinases (Figure 3).

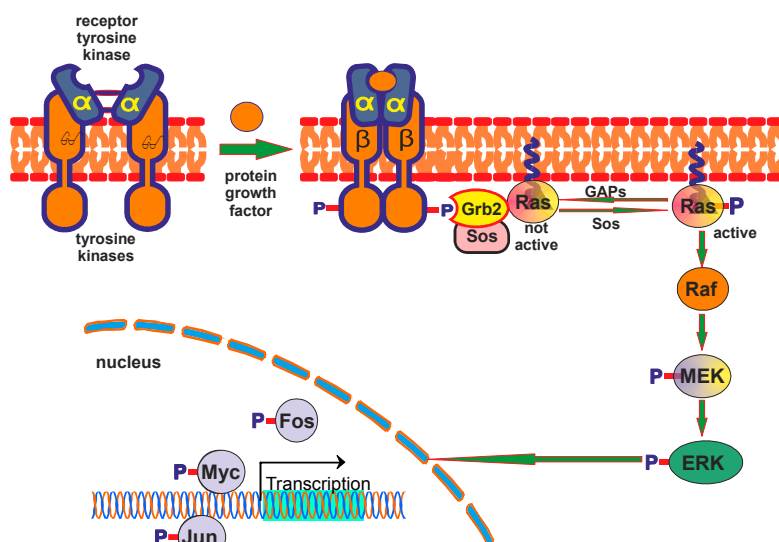


Figure 3. Main elements of the receptor tyrosine kinase pathway.

The activation pathway involves pertussis toxin-sensitive G protein $\beta\gamma$ -subunits, the nonreceptor tyrosine kinase (Src), tyrosine phosphorylated Shc (a molecular docking platform) and a phosphatidylinositol-3 kinase (PI3K) activity [22,48–51]. Moreover, the pathway appears to involve activation of Sos in a multimolecular complex that likely contains Shc and Grb2, $p21^{Ras}$, and $p74^{Raf-1}$ [21,48]. As in growth factor-regulated ERK activation, 5-HT_{1A}R-induced ERK activation is mediated by the small GTPases Ras and Raf [22,48,51,52] and active MAPK/ERK kinase (MEK) [22]. Activation of Ras results in sequential activation of Raf1, which in turn phosphorylates and activates MEK 1 and 2. MEK, a direct upstream protein kinase regulator of ERK, phosphorylates and activates ERK. Additionally, activation of ERK by 5-HT_{1A} receptors in non-neuronal cells can be mediated by the PI3K and phosphatidylcholine-specific PLC in a G protein-dependent manner [22,48,49]. Despite consistent findings in cell systems with heterologous expression of 5-HT_{1A} receptors, effects of 5-HT_{1A} receptors on ERK activity vary in cells of neuronal origin. Consistent findings have shown that 5-HT_{1A} receptor agonists rapidly but transiently increase phosphorylation of ERK in the hypothalamus [53–55], and this effect of 5-HT_{1A} receptors is likely an intermediate step for 5-HT_{1A} receptor-induced elevation of oxytocin, adrenocorticotropin (ACTH), and prolactin [53]. In hippocampal-derived differentiated HN2-5 cells, 5-HT_{1A} agonists increase ERK phosphorylation and activity, an effect that is dependent on the small GTPases Ras and Raf, MEK, and calcium mobilization [23,56].

However, this effect of 5-HT_{1A} receptors was not found in the primary culture of hippocampal neurons [57] or fetal rhombencephalic neurons [58]. In differentiated raphe neurons, 5-HT_{1A} receptors are coupled to a G $\beta\gamma$ subunit-dependent decrease in MEK activity and ERK phosphorylation [59]. In the rat hippocampus *in vivo* 5-HT_{1A} receptor activation decreases ERK phosphorylation [54,60,61] and the upstream activator of ERK1/2, phospho-MEK1/2 [61].

The 5-HT_{1A} receptor can regulate a number of ERK-related effectors, including activation of PI3K [48,49], changes in downstream protein kinases, such as the ribosomal S6 kinase (RSK) [62], stimulation of nuclear factor κ B (NF- κ B) [63], and inhibition of caspase 3 [23,56]. The pathway has been suggested to be involved in neuroprotective mechanisms [23]. ERK may also activate cAMP response element binding (CREB), a widely-studied transcription factor for its gene expression function

and the underlying roles in stress, anxiety, and depression, suggesting that ERK signaling pathway may have important impact in mood-related behaviors [64]. The behavioral effects of the MEK/ERK signaling pathway have been reported in several studies, with MEK inhibitors causing diverse behavioral changes in animals, ranging from hyperactivity, reduced or increased anxiety, and depressive-like behavior [65–68], and MEK inhibitors also block the behavioral effect of antidepressants [69]. It was also demonstrated that the activity of ERK1/2 decreased in the occipital cortex of depressed suicide victims. The MEK/ERK signaling pathways were shown to be involved in transcriptional activation and protein synthesis of neuronal survival and neuroplasticity in depression. Importantly, it was shown that an acute blockade of the MAPK signaling produced a depressive-like phenotype and blocked behavioral actions of antidepressants. Moreover, activation of the MAPK/ERK pathway could inhibit apoptosis by inducing the phosphorylation of Bad (a proapoptotic protein) and increasing the expression of antiapoptotic Bcl-2. Given these observations, MAPK/ERK pathway may be involved in the depression etiopathogenesis and effectiveness of antidepressants [70].

3.4. PI3K and Akt Pathway

Another growth factor-regulated signaling pathway, the PI3K and Akt pathway, can also be regulated by 5-HT_{1A} receptors. When tyrosine kinase receptors are activated by growth factors, they recruit PI3K to activate phosphoinositide-dependent kinase (PDK), which phosphorylates and activates Akt [71]. The PI3K/Akt pathway is classically implicated in the regulation of cell growth, survival, proliferation, and movement [72]. In the mammalian brain, in addition to its functions in neuronal survival and differentiation, several studies have implicated the PI3K/Akt pathway in synaptic plasticity, learning, and memory. It was shown that activation of PI3K/Akt was required for the expression of long-term potentiation (LTP) in the dentate gyrus and CA1 region of the hippocampus. Moreover, pharmacological inhibition of PI3K/Akt significantly impaired the inhibitory avoidance, spatial learning, consolidation, retrieval, and extinction of fear-associated memory [70]. Akt dysregulation has been linked to numerous metabolic diseases including diabetes and obesity, and mental disorders such as schizophrenia and drug abuse. Aberrant Akt function has been progressively linked to a growing number of brain disease states. It has been proposed that Akt may represent a molecular link between diseases driven by insulin resistance (e.g., diabetes) and disorders associated with central monoaminergic disturbances, including depression, schizophrenia and drug abuse [73].

The serotonin receptor class 1 has been reported to activate Erk1/2 and Akt in various cell types [74]. Activation of 5-HT_{1A} receptor in Chinese hamster ovary (CHO) cells resulted in PI3K dependent increased phosphorylation of ERK1/2 and Akt [22]. 5-HT dose-dependently induced activation of Erk1/2 in PC-3, Du145, and LNCaP prostate cancer (PC) cells. Similarly, 5-HT induced phosphorylation of Akt in these cell lines. The action of 5-HT was inhibited to varying degrees by inhibitors of MAPK (U0126) and PI3K (LY294002), as well as by a 5-HT_{1A} receptor antagonist (NAN-190). In addition to proliferation, 5-HT induced migration of PC-3 and Du145 cells, which were alleviated by the aforementioned inhibitors. Regulation of Akt by 5-HT_{1A} receptors in the mammalian brain has not yet been reported; however, some indirect evidence does suggest an effect of 5-HT_{1A} receptors in regulating Akt [24]. It has been shown that 5-HT_{1/7} receptor-selective agonists (5-carboxamidotryptamine and 8-OH-DPAT) can activate Akt in hippocampal primary cultures with similar efficacy and potency as

5-HT, the effects being completely inhibited by the selective 5-HT_{1A} receptor antagonists (p-MPPI and WAY-100635) [57]. It was demonstrated that fluoxetine significantly upregulated expression of the phosphorylated-AKT and ERK1/2 proteins in neural stem cells derived from rats. Besides, expression of phosphorylated-Akt and phosphorylated-ERK1/2 in fluoxetine-treated neural stem cells was effectively blocked by both PI3K inhibitor (LY294002) and MEK inhibitor (PD98059). It was, therefore, suggested that the crosstalk between PI3K/Akt and MAPK/ERK pathways involved Akt and ERK1/2 phosphorylation by fluoxetine treatment [70].

Glycogen synthase kinase 3 (GSK3) is a protein kinase that is primarily phosphorylated and inactivated by Akt [75] and several other protein kinases, such as PKC [76] and PKA [77]. GSK3 is a potential molecular target in several psychiatric disorders, particularly mood disorders, as the mood stabilizer lithium is a selective inhibitor of GSK3 [78,79]. Inhibition of GSK3 by pharmacological or genetic means mimics the effects of antidepressants [80,81] and anti-manic drugs [81,82], whereas impaired regulation of GSK3 results in behavioral abnormalities reminiscent of states of mania and depression [24,83].

Another relevant group of Akt substrates is the Forkhead box O transcription factors (FoxOs). In response to growth factors, active Akt phosphorylates and inactivates FoxOs by exporting them out of the nucleus [84]. In both invertebrate and vertebrate brain, FoxOs can be phosphorylated and inactivated by serotonin via the PI3K/Akt-dependent mechanism [85,86], and the FoxO3a subtype in brain can be inactivated by the antidepressant imipramine [86] and down-regulated by lithium [87]. In addition, mice with FoxO deficiency exhibit antidepressive and anxiolytic behavioral phenotypes [86]. Therefore, regulation of protein substrates by Akt in brain plays a critical role not only in neuronal growth and survival, but also in the maintenance of neuronal activity and behavior.

3.5. Na⁺/H⁺ Exchangers

Na⁺/H⁺ exchangers (NHEs) are expressed in the surface of all mammalian cells, serving to regulate cell volume, intracellular pH, and transepithelial transport of Na⁺ and acid-base equivalents. It has been shown that microinjection of activated Ras or transfection of the Ha-Ras oncogene stimulates NHE activity in fibroblasts. The classical effect of GTP-bound Ras is the activation of the ERK1 and ERK2. This is thought to occur primarily through a linear signaling pathway including Ras-GTP → Raf-1 kinase → MEK (MAPK/ERK kinase) → ERK [51]. Activation of 5-HT_{1A} receptor resulted in the formation of a signaling complex that included activation of Janus kinase (JAK2), Ca²⁺/calmodulin (CaM) and NHE-1, and which involves tyrosine phosphorylation of CaM. It was thus proposed that NHE-1 activation proceeded through the pathway involving 5-HT_{1A} receptor → G(i2)α and/or G(i3)α → Jak2 activation → tyrosine phosphorylation of CaM → increased binding of CaM to NHE-1 → induction of conformational change in NHE-1 that unmasks an obscured proton-sensing and/or proton-transporting region of NHE-1 → activation of NHE-1 [88].

3.6. Nitric Oxide (NO) Production

The 5-HT_{1A} receptor can also regulate the production of NO in some circumstances. It is supposed that putative 5-HT_{1A} receptor in rat ventral prostate cells can stimulate NOS activity [89]. In contrast, 5-HT_{1A} receptors inhibit *N*-methyl-D-aspartate (NMDA)-induced NO production in the adult rat

hippocampus and in human neocortical slices [83,90–92] suggesting that the regulation of NO synthesis by 5-HT_{1A} receptor may be complex and cell-specific [21].

Various inhibitors of nitric oxide synthase (NOS) have been shown to exert antidepressant-like behavioral effect in a variety of animal models. Nitric oxide (NO) plays an important role in the brain, and pharmacological manipulations of the NO pathway will constitute a novel approach for therapeutic applications in the future [93,94]. It was recently found that the antidepressant-like effect of TRIM, a nNOS inhibitor, in the rat FST was partially attenuated by the depletion of endogenous serotonin (with the use of *p*-chlorophenylalanine). It was also suggested—on the basis of the experiments with different 5-HT₁ and 5-HT₂ receptors antagonists—that the antidepressant-like effect of TRIM was mediated, at least in part, by an interaction with 5-HT₂ receptors while non-significant effects were obtained with 5-HT₁ receptors [95].

3.7. Diversity of 5-HT_{1A} Pathways

As already noticed, the 5-HT_{1A} receptor has been found to regulate several channels and transport processes, as well as to activate AD in transfected cells and native tissues (for a review see [21]). In addition, a G protein-independent pathway of 5-HT_{1A} receptor coupling to a smooth inward current (preceding calcium-dependent chloride current) I_{smooth} in *Xenopus laevis* oocytes was also suggested [96].

Raymond *et al.* recently addressed the question about the reasons behind the multiplicity of 5-HT_{1A} receptor signaling. They suggested the ability to activate overlapping pools of G-proteins, activation of distinct signals emanating from G-protein α - and $\beta\gamma$ -subunits, different repertoires of G-proteins and signaling enzymes expressed in cells, ratio of G-protein/effector/and receptor, ligand-specific effects, differential rates of desensitization, and cellular compartmentalization, to name just a few. It was suggested that the variables that affect the multiplicity of signaling from single receptor types are not likely to be manifested as “all or none” effects. The specific coupling of single types of 5-HT receptors to signaling pathways is likely to result from the summation of effects specific to the host cell milieu. One other theoretical possibility is that some signals emanating from 5-HT_{1A} (or other 5-HT) receptors may be regulated by effector pathways activated through non-G-protein-activated pathways [1].

4. 5-HT_{1A} Receptors and Depression

4.1. Serotonin and Depression

Serotonergic system has been implicated in affective illnesses, depression being induced by inhibitors of 5-HT synthesis [97] and tryptophan-depleting diets [98] and being alleviated in the response to SSRIs [99]. Acute tryptophan depletion has been shown to trigger relapse in recovered depressed patients and elicits a depressed mood in normal subjects, while most antidepressant treatments, including SSRIs, increase 5-HT neurotransmission either directly or indirectly. Further, chronic administration of paroxetine in rats with tryptophan depleted levels reduced depression-like behavior [100]. The effects of citalopram have been reported in several studies using rodent in behavioral assays, such as the tail suspension test (TST) and the FST, in which immobility is interpreted as an expression of behavioral despair or entrapment. In the TST, acute administration of citalopram dose-dependently reduced immobility of several mouse strains. In the FST, chronic administration of fluoxetine, another SSRI,

reversed the depressive-like behavior by decreasing immobility and increasing escape attempts of rats [101]. Similar effects were observed following fluoxetine directly infused within the raphe nuclei of mice [102]. Increases in the expression of a particular micro-RNA, named miR-16, has been detected following infusion of fluoxetine in the raphe nuclei, mimicking antidepressive-like behavior [102]. Interestingly, deep brain stimulation of the subthalamic nucleus in naive or dopamine-depleted rats decreased the firing rate of serotonergic neurons in DRN and enhanced immobility in the FST, which was counteracted by chronic administration of citalopram [103].

4.2. Role of Pre- and Postsynaptic 5-HT_{1A} Receptors

Various 5-HT receptor knockout strains have been developed to study the role of serotonin in modulating the stress response. 5-HT_{1A} knockout mice exhibit anxiety-like behaviors, lower hypothalamic-pituitary-adrenal (HPA) response rates, and have reduced adrenal gland weight [104]. Consistently, a transgenic line overexpressing the murine 5-HT_{1A} receptor in the central nervous system under control of its endogenous promoter had reduced anxiety. The transgenic mice revealed typical phenotypic changes indicative of 5-HT_{1A} receptor overactivity—a reduced molar ratio of 5-hydroxyindoleacetic acid to 5-HT in several brain areas and elevated serotonin levels in the hippocampus and striatum [105]. Additionally, mice with complete 5-HT_{1A} receptor knockout (KO), lacking both auto- and heteroreceptors through life, consistently showed increased anxiety in conflict-based anxiety paradigm tasks, while exhibiting decreased behavioral despair in response to stress (higher mobility time in the FST and TST), suggesting that the absence of 5-HT_{1A} receptors could result in an “antidepressant-like” effect [14,106–109]. Since behavioral despair in response to stress is decreased by acute treatment with a number of drugs used to treat depression, this phenotype has been referred to as “antidepressed” [13].

Günther *et al.* [110] examined the impact of postsynaptic 5-HT_{1A}-receptor overexpression in corticolimbic areas (which are thought to be part of the neuronal circuitry of depression [111]) of male and female mice (with female mice displaying higher receptor binding in the distinct brain areas) on the performance in the FST and suggested the involvement of postsynaptic 5-HT_{1A}-receptors in the effects of SSRIs. In the FST untreated male, but not female, overexpressing mice showed an antidepressant-like behavior compared to wild-type mice. Citalopram yielded an antidepressant effect without influencing locomotor activity in overexpressing male but not in wild-type mice. Reboxetine (norepinephrine reuptake inhibitor—NRI) had no antidepressant-like effect in overexpressing mice, but a sex dependent effect in WT mice (antidepressant-like response in female mice). The two partial agonists, buspirone and S 15535, produced no antidepressant-like activity in both genotypes and sexes, but gave aberrant motor effects [110].

It was shown in the transgenic mice model, allowing to independently assess the function of 5-HT_{1A} autoreceptors and heteroreceptors, that suppression of endogenous heteroreceptors was not sufficient to impact anxiety-like behavior [14,112]. Furthermore mice lacking 5-HT_{1A} heteroreceptors throughout the life displayed decreased mobility in the FST, or increased behavioral despair, in adulthood [14]. Suppression of the 5-HT_{1A} heteroreceptors during development leads to an increased behavioral despair in adulthood. In contrast, this phenotype was not observed when heteroreceptor suppression was initiated in adulthood, suggesting that 5-HT_{1A} heteroreceptors act developmentally to establish the circuitry

underlying the behavioral response to forced swim stress without affecting conflict-based anxiety paradigms [112]. It was thus concluded that suppression of 5-HT_{1A} heteroreceptors results in behavioral despair, but not anxiety, while ectopic overexpression in the forebrain of this receptor during development rescues the anxious phenotype of whole brain 5-HT_{1A} KO mice [14] (Table 1).

Richardson-Jones *et al.* [13] elaborated a murine model in which it was possible to specifically modulate 5-HT_{1A} autoreceptor levels without affecting heteroreceptor levels. Transgenic strains with high (1A-high) and low (1A-low) 5-HT_{1A} autoreceptor densities were obtained. 1A-high (but not 1A-low) mice displayed the expected dose-dependent hypothermic response to the 5-HT_{1A} agonist, 8-OH-DPAT. Between mice with low and high 5-HT_{1A} autoreceptor levels no differences in the open field paradigm and the light/dark choice test were observed. Additionally, between 1A-low and 1A-high mice no differences in the TST and FST were observed. However, in the FST 1A-high, but not 1A-low, displayed progressively less mobility or more behavioral despair, upon re-exposure on the second day. Following four weeks of repeated stress, 1A-high and 1A-low mice remained indistinguishable in their total exploration in the open field. However, 1A-high, but not 1A-low mice, displayed less mobility in FST and TST. Thus, while decreasing adult levels of 5-HT_{1A} autoreceptors does alter either conflict-based anxiety or behavioral response to an acute stressor, it results in increased physiological reactivity to stress and appears to elicit a more active response to a repeated stress in a depression-related task (reduced depression-like behavior) [13]. The 1A-low, but not 1A-high, responded to 26 days of fluoxetine treatment in the novelty suppressed feeding paradigm (measuring the latency of a mouse to consume food placed in the middle of a brightly lit, aversive arena). On this basis it was suggested that a decrease in 5-HT_{1A} autoreceptor levels in adulthood, prior to antidepressant treatment, is sufficient to confer responsiveness to fluoxetine in an otherwise treatment-resistant population [13]. It was shown that loss of autoreceptors impacts anxiety in the adult, suggesting that the anxious-like phenotype of the 5-HT_{1A} KO mouse likely results from increased serotonergic signaling from a disinhibited raphe [13,14,112]. Donaldson *et al.* found that decreases in 5-HT_{1A} autoreceptors during development (postnatal days P14-P30, with a maximal reduction of 40% at P21 and return to regular levels by P30) leads to a long-term increases in anxiety levels and decreases levels of social engagement, but does not alter depression-like behaviors [113]. Similarly, it was shown that suppression of endogenous 5-HT_{1A} autoreceptors throughout life is sufficient to increase anxiety-like behavior in the adult [112] and does not impact behavior in the FST in adulthood [14]. However, modulating 5-HT_{1A} autoreceptors in adulthood does not impact anxiety-like behavior and results in lower levels of depression-related behaviors [13,114] (Table 1).

On the basis of their results [14] Garcia-Garcia *et al.* suggested the distinct roles of the two endogenous receptor populations mediating anxious or depression-like phenotypes with autoreceptors impacting the establishment of anxiety-like behavior and with heteroreceptors affecting behavior in the FST, a depression-related test [14]. Different roles of pre- and postsynaptic 5-HT_{1A} receptors was suggested by Albert *et al.* who suggested that reduced activity of post-synaptic 5-HT_{1A} receptor is implicated in anxiety, while an increased transcription of 5-HT_{1A} autoreceptor associates with depression and resistance to chronic SSRI treatment [115]. Consistently, reduced expression of presynaptic 5-HT_{1A} receptors - without altering postsynaptic 5-HT_{1A} receptors was sufficient to evoke antidepressant-like effects in mice [13,116,117] (Table 1).

Table 1. Relationship between 5-HT_{1A} receptor density and behavioral effects.

Behavioural Effect	Auto- and Heteroreceptors	Autoreceptors	Heteroreceptors
Whole life knockout [13,14,104,106–109,112]			
Anxiety	Elevated	Elevated	No impact
Depression	Lower	No impact	Elevated
Knock-down between 14 and 30 days (40%) [113]			
Anxiety	- *	Long-term increase	-
Depression	-	No effect	-
Adulthood [9,13,114]			
Anxiety	-	No effect	-
Depression	-	Diminished	-
Reduced activity			
Anxiety	-	-	Elevated [115]
Depression	-	Diminished [13,116,117]	-
Overexpression			
Anxiety	Diminished [105,118]	-	-
Depression	-	Elevated [110]	-
Increased transcription			
Depression	-	Increased [115]	-

* No data.

An interesting conclusion may be drawn from two novel 5-HT_{1A} receptor agonists F15599 [55] and F13714 [119]. Detailed analysis of their *in vitro*, *in vivo*, electrophysiological, and neurochemical profile indicate that, contrary to F13714, F15599 preferentially activates post- *versus* pre-synaptic 5-HT_{1A} receptors [120] and influences frontal cortex pyramidal neuron electrical activity at doses that are an order of magnitude lower than those that inhibit raphe neuron electrical activity [121]. The compounds exhibited a potent (similar) activity in the FST and in conditioned stress-induced ultrasonic vocalization in rats, despite the fact that F13714 had an *in vitro* potency *ca.* two orders of magnitude greater than F15599 (K_i 5-HT_{1A} CHO cells 10.40 and 8.57, respectively) and substantial activity of F13714 at presynaptic 5-HT_{1A} receptors [122]. Additionally, F15599 had a remarkable capacity (as compared to F13714) to reverse phencyclidine (non-competitive antagonist of NMDA-glutamatergic receptors) induced memory/cognition deficits and had a lower propensity than F13714 to induce serotonergic syndrome (lower lip retraction, forepaw treading, flat body posture) [122]. F15599 exhibited lower than F13714 efficacy for ERK phosphorylation (pEC₅₀ 7.81 and 9.07, respectively) and cAMP accumulation inhibition (pEC₅₀ 6.46 and 8.67, respectively). Both compounds activated G_{ai} and G_{ao} subunits in HeLa-h5-5-HT_{1A} cell membranes, although with different efficacy. It was, thus, suggested that distinct signaling profiles (Figure 4) and functional selectivity for specific receptor activation responses of F15599 underlined its favorable pharmacological profile [55]. It is, however, to be determined which transduction pathway—Activation of pre- or post-synaptic 5-HT_{1A} receptors, ERK phosphorylation, cAMP inhibition, G_{ai} or G_{ao} activation—Is connected to a particular behavioral syndrome. Interestingly, activation of presynaptic 5-HT_{1A} receptors does not influence F13714 antidepressant activity, as compared to F15599.

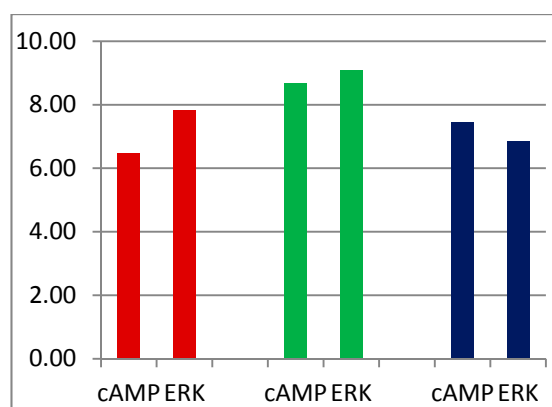


Figure 4. Pharmacological profile (pEC₅₀ for cAMP inhibition and ERK1/2 phosphorylation on the Y axis) of F15599 (brown), F13714 (green), and 5-HT (dark blue) [55].

4.3. Antidepressant Treatment and 5-HT_{1A} Receptors

According to pharmacological studies, pre-synaptic and post-synaptic 5-HT_{1A} receptor activation appears to be involved in anxiolytic and antidepressant effects, respectively [9]. Alterations in 5-HT_{1A} receptor levels are commonly observed in depressed individuals. Reduced somatodendritic and postsynaptic 5-HT_{1A} receptor numbers or affinity have been reported in some post-mortem studies of suicide victims, a result consistent with well-replicated positron emission tomography (PET) analyses demonstrating reduced 5-HT_{1A} receptor binding potential in diverse regions such as the dorsal raphe, medial PFC, amygdala, and hippocampus [123].

Post-synaptic 5-HT_{1A} receptors are reduced in several cortical regions in depression and anxiety, while 5-HT_{1A} autoreceptors are increased in depression. Elevated 5-HT_{1A} autoreceptor expression would tend to reduce the activity of 5-HT neurons, while reduced post-synaptic 5-HT_{1A} receptors would result in a blunted behavioral response to 5-HT [10,14]. Hence, post-mortem and genetic studies have shown that individuals with elevated density or activity of 5-HT_{1A}-autoreceptors were more susceptible to mood disorders and suicide, and respond poorly to antidepressants [124–126]. However, PET studies using [¹¹C]-WAY-100635 to label 5-HT_{1A} receptors have shown no case-control differences, increases, nor decreases of the binding potential of 5-HT_{1A} receptors in major depressive disorder (MDD) patients. A lowered 5-HT_{1A} receptor binding potential has also been found in recovered major depressives compared to controls, which has led to the suggestion that a low 5-HT_{1A} receptor density may confer vulnerability to MDD [2]. In this regard, MDD may be associated with a reduced post-synaptic 5-HT_{1A} receptor-mediated function, as suggested by PET scan studies [122,127], although this abnormality persists after clinical remission [128].

Rodent studies have shown that pre-synaptic 5-HT_{1A} (and to a lower extent, 5-HT_{1B}) autoreceptors play a major detrimental role in antidepressant treatments, due to the activation of negative feedback mechanisms operating in 5-HT neurons following the increase in extracellular 5-HT evoked by SERT blockade. Hence, excitatory inputs increase 5-HT release in the midbrain raphe. The excess 5-HT activates dendritic 5-HT_{1A} autoreceptors, hyperpolarizes 5-HT neurons and, thus, opposes the incoming excitatory inputs. Antidepressant drugs evoke a pharmacological overactivation of this physiological feedback mechanism by markedly increasing extracellular 5-HT in the raphe nuclei (which contain the largest SERT density in rodent and human brain). Thus, the indirect 5-HT_{1A} receptor activation

by SSRI and selective NRI (and by monoaminoxidase inhibitors) reduces serotonergic activity and 5-HT release in forebrain, thereby attenuating the 5-HT elevation produced by SERT blockade in corticolimbic networks thus limiting the activation of postsynaptic 5-HT receptors responsible for clinical antidepressant effects [2,129]. Recent observations with PET suggest the existence of a similar negative feedback mechanism in primate and human brains. Thus, a single, clinically-relevant dose of escitalopram increased 5-HT release in the raphe nuclei and reduced it in the projection areas [130].

This negative feedback mechanism is involved in the delayed action—and possibly, limited efficacy—of antidepressant drugs. The preferential blockade of presynaptic 5-HT_{1A} receptors by pindolol (mixed β -adrenoceptor/5-HT_{1A} receptor antagonist) accelerates the clinical effects of SSRIs [131–133]. Pindolol partially blocks the SSRI-induced, 5-HT_{1A} receptor-mediated negative feedback and augments forebrain 5-HT release, mimicking the 5-HT_{1A} autoreceptor desensitization produced by chronic antidepressant treatment [134].

Chronic treatment with antidepressant drugs desensitizes pre-synaptic 5-HT_{1A} autoreceptors, which reduces the efficacy of the autoreceptor-mediated negative feedback and enables a normalization of 5-HT release, thus allowing a greater activation of postsynaptic 5-HT receptors responsible for clinical antidepressant action.

Studies in rats treated chronically with SSRIs have shown an initial decrease of raphe firing at the beginning of treatment, with firing rates recovering to baseline following chronic treatment and 5-HT_{1A} autoreceptor desensitization [135]. The long time required for SSRIs' clinical efficacy onset (several weeks) may be attributed to the necessity of 5-HT_{1A} autoreceptor desensitization or/and to the time required to evoke plastic changes in certain brain areas involving protein synthesis or trophic actions. Additionally, repeated treatment with 5-HT_{1A} agonists desensitizes pre-synaptic 5-HT_{1A} receptors in the raphe nuclei, which disengages 5-HT neurons from autoreceptor-mediated inhibition. As a consequence, 5-HT neurons are activated by chronic treatment with 5-HT_{1A} agonists, and this counteracts the 5-HT deficit in depression [136,137]. 5-HT_{1A} antagonist can improve the efficacy of SSRIs by either blocking inhibitory 5-HT_{1A} autoreceptors or could have antidepressant-like activity by triggering post-synaptic 5-HT_{1A} receptors and/or producing a faster desensitization of 5-HT_{1A} autoreceptors. In line with this, the selective inactivation of presynaptic 5-HT_{1A} receptors by genetic or molecular means (siRNA) is sufficient to evoke robust antidepressant-like effects in rodents [9].

Several 5-HT_{1A} receptor agonists show antidepressant activity in various animal models, such as TST and FST [135,138]. 5-HT_{1A} receptor KO mice display increased anxiety-related behavior, which, unlike in their wild-type counterparts, cannot be rescued with antidepressant drug treatment [123]. The activation of post-synaptic hippocampal 5-HT_{1A} receptors plays an important role in MDD. Chronic antidepressant treatments tonically activate hippocampal 5-HT_{1A} receptors [123,135]. Furthermore, genetic studies indicate that post-synaptic 5-HT_{1A} receptors are sufficient for antidepressant-like effects in rodents [112]. Antidepressant effects of some 5-HT_{1A} drugs may be attributed to the activation of post-synaptic 5-HT_{1A} receptor sites, an effect likely linked to their therapeutic action [139,140]. Lithium, valproate, SSRIs, tricyclic antidepressants (TCAs), and other treatments, such as electroconvulsive shock therapy, all increase post-synaptic 5-HT_{1A} receptor signaling through either direct or indirect effects [123]. These observations, together with the antidepressant properties of 5-HT_{1A} agonists in preclinical tests, have led to the suggestion of post-synaptic 5-HT_{1A} agonists as a new class of antidepressants [135]. Most 5-HT_{1A} agonists developed so far—and in particular, the azapirones—show

full agonist properties at pre-synaptic 5-HT_{1A} autoreceptors and are partial agonists at post-synaptic 5-HT_{1A} receptors, which results in a reduced tone on post-synaptic 5-HT_{1A} receptors after the administration of 5-HT_{1A} agonists [2]. Under conditions of increased serotonin release, the occupancy of post-synaptic 5-HT_{1A} receptors by a partial agonist may actually reduce 5-HT_{1A} receptor transmission by blocking the direct effects of serotonin while inducing only a partial agonist effect instead [122].

Despite the initial hopes placed on the clinical use of 5-HT_{1A} receptor agonists as antidepressant drugs, these agents achieved little clinical success, partly due to their limited clinical efficacy and to the widespread occurrence of gastrointestinal side effects. Several azapirones, such as buspirone, gepirone, ipsapirone or tandospirone, showed antidepressant-like efficacy in preclinical studies, and clinical efficacy in open-label or placebo-controlled trials. The failure of azapirones in MDD may be related to their preferential presynaptic activity [135,141]. Classical 5-HT_{1A} receptor agonists, and particularly azapirones (buspirone, gepirone, *etc.*) are full agonists at raphe 5-HT_{1A} receptors and partial agonists at post-synaptic hippocampal 5-HT_{1A} receptors [142]. However, some new agents with chemical structures different from the azapirones (e.g., F15599) display preferential agonist actions at post-synaptic 5-HT_{1A} receptors [55,143].

5-HT_{1A} receptor antagonists may improve the clinical effects of antidepressants by preventing the 5-HT_{1A} autoreceptor-mediated negative feedback [144]. The antagonists may thus mimic the 5-HT_{1A} autoreceptor desensitization effect of chronic SSRIs administration, thus augmenting the effect of SSRIs on forebrain extracellular 5-HT concentration. In this respect, selective antagonists, preferentially blocking pre-synaptic *vs.* post-synaptic 5-HT_{1A} sites, would be required [2,145,146].

It was suggested [9] that 5-HT_{1A} partial agonism, combined with 5-HT reuptake inhibition, can produce antidepressant-like effects. These observations have led to the development of new antidepressant drugs blocking the serotonin transporter (SERT) and having partial agonist activity at 5-HT_{1A} receptors, in order to evoke a higher increase in extracellular 5-HT: Vilazodone, approved in 2011 by the FDA for use in the treatment of MDD, and vortioxetine (LuAA21004, in development) [147,148]. Similarly flibanserin, DU-125530, and OPC-14523 have reached late stage clinical development as antidepressant drugs [9].

Mayorga *et al.* showed that fluoxetine and paroxetine failed to ameliorate immobility behavior in 5-HT_{1A} KO mice exposed to a stressor, suggesting that 5-HT_{1A} receptor activation is a necessary feature of the antidepressive response [149]. It was, however, shown that 5-HT_{1A} KO mice respond to TCAs, but not to the SSRI fluoxetine, in the TST and the novelty-suppressed feeding test, suggesting that the 5-HT_{1A} receptors are a critical component in the mechanism of action of SSRIs, but not TCAs [148,150]. It should also be noted that according to clinical data, full 5-HT_{1A} blockade neither enhances nor cancels the antidepressant effect of fluoxetine in MDD patients [139], suggesting the involvement of other 5-HT receptors (e.g., 5-HT₄ receptor) [151].

Recent studies suggested that neurogenesis is involved in the action of antidepressants [152]. Neurogenesis, the process of neuronal stem cell proliferation, differentiation and survival, has also been thought to occur in humans, at a slow, but detectable rate [150]. Santarelli *et al.* [153] demonstrated the involvement of antidepressants in adult hippocampal neurogenesis. In addition, this study showed that 5-HT_{1A} knockout mice did not exhibit neurogenesis or respond behaviorally to SSRI treatment, implicating these receptors in antidepressant-mediated neurogenesis. The antidepressant effects of 5-HT_{1A} receptor stimulation may be related to the induction of hippocampal neurogenesis, a common feature of

antidepressant treatments [154], which depends on 5-HT_{1A} receptor activation [150], but also the functional remodeling of corticolimbic circuits involved in MDD [122]. It was thus suggested that the treatment with antidepressants could lead to adaptive changes such as neurogenesis over a period of weeks, which may account for the delayed therapeutic effect of these drugs [155].

As with the 5-HT_{1A} receptors, acute SSRI administration activates terminal 5-HT_{1B} receptors, thus reducing 5-HT synthesis and release. Likewise, chronic administration of SSRI also results in desensitization of terminal 5-HT_{1B} autoreceptors, suggesting that plasticity in both the 5-HT_{1A}- and 5-HT_{1B}-mediated autoregulatory function may be important in the therapeutic profile of SSRI [156,157]. Moreover, as observed with 5-HT_{1A} receptors, the administration of 5-HT_{1B} receptor antagonists augments the neurochemical and behavioral effects of SSRI [158,159]. The effects of SSRIs have also been analyzed in genetic rodent models of depressive-like behaviors, such as in mice lacking the 5-HT_{1B} receptor. The acute administration of citalopram or paroxetine failed to decrease the immobility in the FST of the 5-HT_{1B} (KO) compared to WT mice, suggesting that antidepressant effects of SSRI depend on activation of the 5-HT_{1B} receptor [6]. Interestingly, the effects of 5-HT_{1B} antagonists on pre-synaptic 5-HT function are additive to those of 5-HT_{1A} antagonists, indicating a diversity of mechanisms to augment the effects of SSRI [160]. Mice lacking both 5-HT_{1A/1B} autoreceptors exhibit an increased 5-HT transmission also associated to anxiety-like behavior. While acute paroxetine failed to reverse anxiety-like behavior, its chronic administration still led to anxiolytic effects in the elevated plus maze [6].

5. Models of Depression Involved Circuits

The current limitation of SSRI/SNRI treatments most likely derives from the poor knowledge of the pathophysiology of major depression, in common with other psychiatric disorders. The lack of a unifying theory on the cause of depression, together with the partial success of antidepressant drugs enhancing serotonergic (and to a lesser success, noradrenergic) function, has dominated the antidepressant drug development so far. Numerous clinical and preclinical studies indicate that disturbances in serotonergic activity may be associated with major depression. Noradrenergic and dopaminergic neurotransmission has also been implicated, although the exact changes in these monoamine systems are unknown. Historically, a functional hypoactivity of these systems has been assumed in depression. This view was mainly based on the observation that antidepressant drugs increase monoaminergic function (particularly serotonin and norepinephrine). One commonality of these ascending monoaminergic systems is that their cell bodies are located in the brain stem and that their activity is tightly controlled by the PFC, a cortical area where metabolic abnormalities have been reported in depressive patients. Thus, the raphe nuclei, the locus coeruleus, and the ventral tegmental area, where the cell bodies of ascending serotonergic, noradrenergic, and dopaminergic neurons are located, respectively, receive dense afferents from dorsal and ventral parts of the medial PFC in rodents, which are equivalent to dorsal and ventral cingulate as in primate and human brain. The treatment with antidepressant drugs would restore monoamine function in cortical and limbic areas, thus improving depressive symptoms. Based on these, Artigas has shown a schematic representation (not included) of the functional connectivity between the medial prefrontal cortex and the dorsal and median raphe nuclei of the midbrain [2].

Albert *et al.* proposed a simplified model of raphe and PFC circuitry involvement in anxiety and depression (Figure 5) [161]. It was suggested that there are two major sub-populations of heteroreceptors in PFC: 5-HT_{1A} receptors on pyramidal glutamatergic neurons and on GABA interneurons that inhibit the activity of both neuron types [162]. In rodent PFC, ~50% of pyramidal neurons and ~25% of GABAergic interneurons express 5-HT_{1A} receptors [163], and this proportion is even higher (up to 80%) in upper cortical layers of primate and human PFC [162]. It should also be noted that 5-HT_{1A} receptors are abundantly coexpressed (in *ca.* 80%) with excitatory 5-HT_{2A} receptors in rodent PFC [161]. 5-HT_{2A} receptors have 10-fold lower affinity for 5-HT and with maturation 5-HT_{1A} receptor inhibition predominates over 5-HT_{2A} excitatory function. In this model the activity of the GABA interneurons exhibits inhibitory activity on glutamatergic pyramidal neurons while 5-HT_{1A} heteroreceptors diminish the GABAergic activity. In the anxiety model, under normal conditions both 5-HT_{1A} heteroreceptors on pyramidal and interneurons are engaged, resulting in a balance between 5-HT_{1A}-mediated inhibition and dys-inhibition on pyramidal neurons. On the basis of evidence from human imaging studies, it was suggested that different sub-populations of PFC neurons with different targets mediate anxiety *vs.* depression behavior. Due to these differences, the serotonin circuitry model for depression involves similar components but is slightly different from the anxiety model. First, opposite to anxiety, activation of the pyramidal neuron is associated with reduced depression and increased resilience. Second, it was proposed that the 5-HT_{2A} receptor is weakly active in the mature pyramidal neurons controlling depression. The model predicts elevated anxiety at the low and high serotonin levels and elevated depression at the lack of serotonin and its low levels [162].

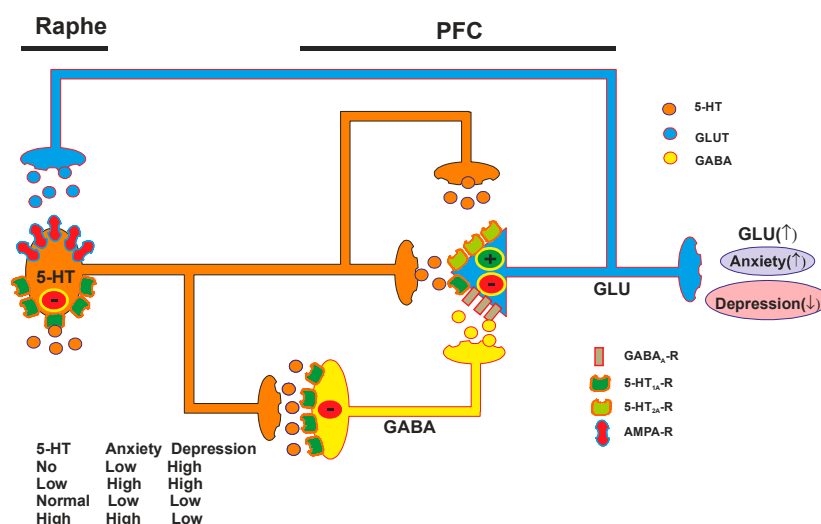


Figure 5. Adult 5-HT raphe-PFC circuitry in anxiety and depression model (according to Albert *et al.*) [161]. The model shows 5-HT neurons (brown) projecting to prefrontal cortex GABAergic interneurons (yellow) and glutamatergic pyramidal neurons (blue) with transmitter release illustrated as small circles of the same colors. Receptors: 5-HT_{1A} (green), 5-HT_{2A} (light green), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-glutamate (purple) and GABA_A receptors (blue). The response in the target neurons (red ovals): stimulatory (+), inhibitory (−). (↑)—elevation, (↓)—decrease. 5-HT_{1A} heteroreceptors and GABA_A receptors reduce the activity of the pyramidal neurons while 5-HT_{2A} receptors activate the neurons.

The model was shown to fit with the genetic model where mutants with reduced or eliminated 5-HT show depression phenotypes, while suppression of 5-HT_{1A} autoreceptors in adults to increase the 5-HT activity reduces depressive behavior. Reduced interneuron 5-HT_{1A} receptors would enhance interneuron firing and inhibit pyramidal firing, leading to a depression phenotype similar to the low or no 5-HT condition. Suppression of pyramidal 5-HT_{1A} heteroreceptors (using the CaMKII α promoter to target pyramidal neurons) would be predicted to increase pyramidal neuron firing and display an anxiety phenotype rather than depression. Over-expression of 5-HT_{1A} autoreceptors would reduce 5-HT activity leading to a hyperactive anxiety circuit by preferentially relieving inhibition of pyramidal neurons. On the other hand, global knockout of all 5-HT_{1A} receptors would allow for hyper-activation of raphe neurons (due to absence of 5-HT_{1A} autoreceptor) and activation of stimulatory pyramidal 5-HT_{2A} receptors that is not antagonized by pyramidal 5-HT_{1A} receptors, leading to increased anxiety. Pyramidal 5-HT_{2A} receptor activation may also actively suppress interneuronal GABA-mediated inhibition via activation of protein kinase C. Consistent with a pro-anxiety role, mice lacking 5-HT_{2A} receptors display reduced anxiety that is reversed by cortical re-expression of 5-HT_{2A} receptors [162].

It was shown that numerous antidepressants and antipsychotic drugs bind with relatively high affinity at 5-HT_{2A} receptors and several clinical studies have shown that atypical antipsychotics augment the clinical response in treatment-resistant patients. The effect was suggested to be due to their ability to occupy 5-HT₂ receptors, and more specifically to block 5-HT_{2A}-mediated response. The increased extracellular 5-HT concentration produced by SERT blockade activates excitatory 5-HT_{2A} receptors and inhibitory 5-HT_{1A} receptors (Figure 6A). Given the large co-expression of 5-HT_{1A} and 5-HT_{2A} receptors in the neocortex [164] blockade of 5-HT_{2A} receptors (or atypical antipsychotic drugs) blocks excitatory effects of 5-HT on pyramidal neurons. That may enhance inhibitory 5-HT_{1A} receptor-mediated neurotransmission in cortical and limbic areas, an effect likely linked to the antidepressant activity (Figure 6B). This effect may reduce a potential hyperactivity in ventral cingulate areas and normalize the functional connectivity between ventral and dorsal PFC areas and with limbic areas and monoamine systems [2].

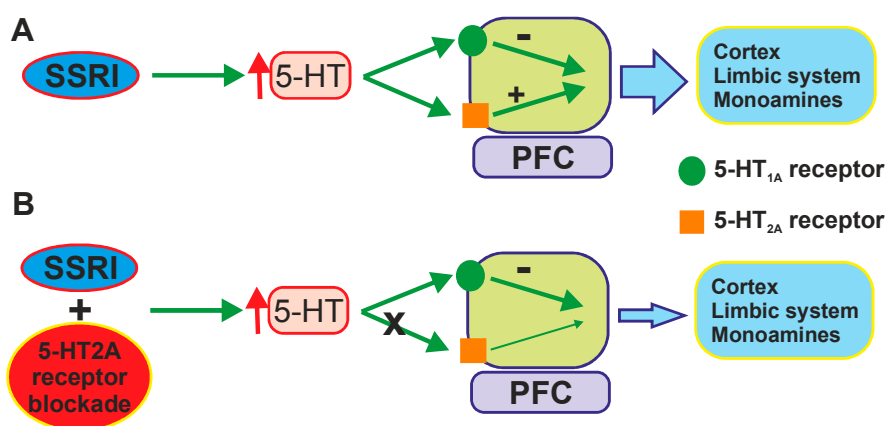


Figure 6. The potential mechanism underlying the augmenting effects of 5-HT_{2A} receptor blockade. (A) Increased extracellular 5-HT concentration may activate limbic monoamine system; (B) Blockade of activating 5-HT_{2A} receptors may reduce a potential hyperactivity of the monoamine system (according to [2]).

Bevilacqua *et al.* have suggested a functional interaction between SERT and the 5-HT_{2B} receptor—a receptor implicated in 5-HT-dependent phenotypes, including impulsivity, aggressivity and suicidality [165]. *Ex vivo* studies have indicated that 5-HT_{2B} receptors might participate in the control of SERT in raphe neurons [166], while *in vivo* studies further confirmed that 5-HT_{2B} receptors contribute to the behavioral and physiological effects of the SERT-targeting 5-HT releasers, 3,4-methylenedioxymethamphetamine (MDMA, the club-drug ecstasy) and dexfenfluramine [167–169]. Diaz *et al.* [170] reported that the acute response to SSRIs in the FST, a classical test for antidepressant activity, is absent in mice lacking 5-HT_{2B} receptors. Conversely, a 5-HT_{2B} receptor agonist induced an antidepressant-like action in the FST, suggesting that this receptor is required for acute SSRI effects and might modulate serotonergic tone. It was also shown that long-term behavioral and neurogenic SSRI effects were abolished after either genetic or pharmacological inactivation of 5-HT_{2B} receptors. Conversely, direct agonist stimulation of 5-HT_{2B} receptors induced an SSRI-like response in behavioral and neurogenic assays suggesting that antidepressant effects of SSRI depend on activation of the 5-HT_{2B} receptor [169,171].

Another candidate for mediating the antidepressant-like effects of SSRIs is the 5-HT_{2C} receptor. 5-HT_{2C} receptor agonists show antidepressant-like effects in multiple animal models (both acute and chronic) of depression [172]. For example, 5-HT_{2C} receptor agonists decrease immobility time and increase swimming time in the FST in rats in a manner comparable to SSRIs. The effects of the 5-HT_{2C} receptor agonists and SSRIs in the rat forced swim test are antagonized by the 5-HT_{2C} antagonists [173] consistent with the role for 5-HT_{2C} receptors in mediating antidepressant-like effects of 5-HT_{2C} receptor agonists and SSRIs (recently Rosenzweig-Lipson *et al.* have shown that novel 5-HT_{2C} receptor agonist WAY-163909 exhibited antidepressant-like effects in rodents).

6. Conclusions

5-HT_{1A} receptors have, for long time, been considered as interesting targets for antidepressant drugs. It was thus postulated that postsynaptic 5-HT_{1A} agonists could form a new class of antidepressant drugs. It was also stated that most 5-HT_{1A} agonists developed so far—and in particular, the azapirones—show full agonist properties at pre-synaptic 5-HT_{1A} autoreceptors and are partial agonists at post-synaptic 5-HT_{1A} receptors, which results in a reduced tone on post-synaptic 5-HT_{1A} receptors after the administration of 5-HT_{1A} agonists.

There is a, more or less, consistent view concerning the role of 5-HT_{1A} receptors in depression and anxiety, with autoreceptors impacting the establishment of anxiety and heteroreceptors affecting depression-like tests. Results with 5-HT_{1A} receptor KO animals have shown that 5-HT_{1A} receptor KO mice exhibit increased anxiety-like behavior and decreased levels of depression. In line with that 5-HT_{1A} receptor overexpressing mice exhibit reduced anxiety. It was shown that 5-HT_{1A} autoreceptor down-regulation resulted in antidepressant-like effects, and increased transcription was associated with depression, resistance to chronic SSRIs treatment, and mice lacking heteroreceptors throughout life exhibited increased levels of depression.

It was suggested that the anxious-like phenotype of the 5-HT_{1A} KO mouse likely results from increased serotonergic signaling from a disinhibited raphe. While decreasing adult levels of 5-HT_{1A} autoreceptors does alter either conflict-based anxiety or behavioral response to an acute stressor, it may

result in increased physiological reactivity to stress and appears to elicit more active responses to a repeated stress in a depression-related task (reduced depression-like behavior).

It was shown, that although 5-HT_{1A} KO mice do not respond to the SSRI fluoxetine, they respond to TCAs (TST and the novelty-suppressed feeding test), suggesting that the 5-HT_{1A} receptors are a critical component in the mechanism of action of SSRIs, but not TCAs. According to clinical data, full 5-HT_{1A} blockade neither enhances nor cancels the antidepressant effect of fluoxetine in MDD patients suggesting the involvement of other 5-HT receptors (e.g., 5-HT₄ receptor).

Agonist-directed trafficking for 5-HT_{1A} receptor signaling exists in rat native brain tissue. Thus, the receptors have the ability to activate overlapping pools of G-proteins, activate distinct signals emanating from G-protein α - and $\beta\gamma$ -subunits and differing repertoires of G-proteins and signaling enzymes expressed in cells. It may express different ratios of receptor/G-protein/effector, ligand-specific effects, differential rates of desensitization, and cellular compartmentalization, as well as activate effector pathways through non-G-protein-activated pathways. The human 5-HT_{1A} receptors, located in different brain regions despite similar [³H]-8-OH-DPAT binding profiles, present a different functional pharmacology. Moreover, receptor trafficking appears different at pre- and post-synaptic sites, with pre-synaptic 5-HT_{1A} receptors showing more marked adaptive processes, including desensitization and down-regulation [156,174,175].

The 5-HT_{1A} receptor may still be considered as valuable target for antidepressant drugs. New mixed 5-HT_{1A} receptor ligands/SERT inhibitors seem to possess interesting pharmacological profiles. Although identifying the real molecular and brain-specific target for 5-HT_{1A} receptor ligands may be a very complicated task due to the receptor signal transduction pathway complexity, it may result in better targeting, raising hope for more effective medicines for various pathologies.

It should also be noted that 5-HT_{1A} receptor cooperates with other transduction systems (like 5-HT_{1B} and 5-HT_{2A/2B/2C} receptors, GABA, glutamine) with relying its antidepressant and/or anxiolytic activity. Most probably additional serotonergic targets like 5-HT_{1B}, 5-HT_{2B} or 5-HT₄₋₇ receptors should also be considered. 5-HT_{1B} receptor antagonists administered alone or with antidepressants have been shown to be effective in preclinical models of depression; the activation of 5-HT_{1B} heteroreceptors induces antidepressant-like behavior. As with the 5-HT_{1A} receptors, acute SSRI administration activates terminal 5-HT_{1B} receptors, thus reducing 5-HT synthesis and release. Likewise, chronic administration of SSRI also results in desensitization of terminal 5-HT_{1B} autoreceptors, suggesting that plasticity in both the 5-HT_{1A}- and 5-HT_{1B}-mediated autoregulatory function may be important in the therapeutic profile of SSRI. Mice lacking both 5-HT_{1A/1B} autoreceptors exhibit an increased 5-HT transmission also associated to anxiety-like behavior. 5-HT_{2B} receptors are also expressed by raphe serotonergic neurons. SSRI-induced increase in hippocampal extracellular 5-HT concentration is strongly reduced in the absence of functional 5-HT_{2B} receptors and selective 5-HT_{2B} agonists may mimic SSRI responses. 5-HT_{2B} receptors were shown to be required for the therapeutic actions of SSRIs. 5-HT₄₋₇ receptors are also considered as new molecular targets for antidepressant drugs.

Acknowledgments

The study was supported by the Polish-Norwegian Research Programme operated by the National Centre for Research and Development under the Norwegian Financial Mechanism 2009–2014 in the frame of Project PLATFORMex (Pol-Nor/198887/73/2013).

Author Contributions

All the authors were involved in the literature search, discussion and text preparation and their contribution can be evaluated as equal.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Raymond, J.R.; Mukhin, Y.V.; Gelasco, A.; Turner, J.; Collinsworth, G.; Gettys, T.W.; Grewal, J.S.; Garnovskaya, M.N. Multiplicity of mechanisms of serotonin receptor signal transduction. *Pharmacol. Ther.* **2001**, *92*, 179–212.
2. Artigas, F. Developments in the field of antidepressants, where do we go now? *Eur. Neuropsychopharmacol.* **2015**, *25*, 657–670.
3. Jacobs, B.L.; Azmitia, E.C. Structure and function of the brain serotonin system. *Physiol. Rev.* **1992**, *72*, 165–229.
4. Artigas, F. Serotonin receptors involved in antidepressant effects. *Pharmacol. Ther.* **2013**, *137*, 119–131.
5. Adell, A.; Celada, P.; Abellan, M.T.; Artigas, F. Origin and functional role of the extracellular serotonin in the midbrain raphe nuclei. *Brain Res. Brain Res. Rev.* **2002**, *39*, 154–180.
6. Beaudoin-Gobert, M.; Sgambato-Faure, V. Serotonergic pharmacology in animal models: From behavioral disorders to dyskinesia. *Neuropharmacology* **2014**, *81*, 15–30.
7. Valdizan, E.M.; Castro, E.; Pazos, A. Agonist-dependent modulation of G-protein coupling and transduction of 5-HT_{1A} receptors in rat dorsal raphe nucleus. *Int. J. Neuropsychopharmacol.* **2010**, *13*, 835–843.
8. Fiorino, F.; Severino, B.; Magli, E.; Ciano, A.; Caliendo, G.; Santagada, V.; Frecentese, F.; Perissutti, E. 5-HT (1A) receptor: An old target as a new attractive tool in drug discovery from central nervous system to cancer. *J. Med. Chem.* **2014**, *57*, 4407–4426.
9. Celada, P.; Bortolozzi, A.; Artigas, F. Serotonin 5-HT_{1A} receptors as targets for agents to treat psychiatric disorders: Rationale and current status of research. *CNS Drugs* **2013**, *27*, 703–716.
10. Albert, P.R.; Le, F.B.; Millar, A.M. Transcriptional dysregulation of 5-HT_{1A} autoreceptors in mental illness. *Mol. Brain* **2011**, *4*, 21–24.
11. Hervas, I.; Queiroz, C.M.; Adell, A.; Artigas, F. Role of uptake inhibition and autoreceptor activation in the control of 5-HT release in the frontal cortex and dorsal hippocampus of the rat. *Br. J. Pharmacol.* **2000**, *130*, 160–166.

12. Bang, S.J.; Jensen, P.; Dymecki, S.M.; Commons, K.G. Projections and interconnections of genetically defined serotonin neurons in mice. *Eur. J. Neurosci.* **2012**, *35*, 85–96.
13. Richardson-Jones, J.W.; Craige, C.P.; Guiard, B.P.; Stephen, A.; Metzger, K.L.; Kung, H.F.; Gardier, A.M.; Dranovsky, A.; David, D.J.; Beck, S.G.; *et al.* 5-HT_{1A} autoreceptor levels determine vulnerability to stress and response to antidepressants. *Neuron* **2010**, *65*, 40–52.
14. Garcia-Garcia, A.L.; Newman-Tancredi, A.; Leonardo, E.D. 5-HT (1A) receptors in mood and anxiety: Recent insights into autoreceptor *versus* heteroreceptor function. *Psychopharmacology* **2014**, *231*, 623–636.
15. Pauwels, P.J. 5-HT 1B/D receptor antagonists. *Gen. Pharmacol.* **1997**, *29*, 293–303.
16. Hoyer, D.; Hannon, J.P.; Martin, G.R. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.* **2002**, *71*, 533–554.
17. Malleret, G.; Hen, R.; Guillou, J.L.; Segu, L.; Buhot, M.C. 5-HT_{1B} receptor knock-out mice exhibit increased exploratory activity and enhanced spatial memory performance in the Morris water maze. *J. Neurosci.* **1999**, *19*, 6157–6168.
18. Malagie, I.; David, D.J.; Jolliet, P.; Hen, R.; Bourin, M.; Gardier, A.M. Improved efficacy of fluoxetine in increasing hippocampal 5-hydroxytryptamine outflow in 5-HT_{1B} receptor knock-out mice. *Eur. J. Pharmacol.* **2002**, *443*, 99–104.
19. Tatarczynska, E.; Klodzinska, A.; Stachowicz, K.; Chojnacka-Wojcik, E. Effects of a selective 5-HT_{1B} receptor agonist and antagonists in animal models of anxiety and depression. *Behav. Pharmacol.* **2004**, *15*, 523–534.
20. Chenu, F.; David, D.J.; Leroux-Nicollet, I.; Le, M.E.; Gardier, A.M.; Bourin, M. Serotonin_{1B} heteroreceptor activation induces an antidepressant-like effect in mice with an alteration of the serotonergic system. *J. Psychiatry Neurosci.* **2008**, *33*, 541–550.
21. Raymond, J.R.; Turner, J.H.; Gelasco, A.K.; Ayiku, H.B.; Coaxum, S.D.; Arthur, J.M.; Garnovskaya, M.N. 5-HT Receptor Signal Transduction Pathways. In *The Serotonin Receptors. From Molecular Pharmacology to Human Therapeutics*; Roth, B.R., Ed.; Humana Press: Toyota, NJ, USA, 2006.
22. Hsiung, S.C.; Tamir, H.; Franke, T.F.; Liu, K.P. Roles of extracellular signal-regulated kinase and Akt signaling in coordinating nuclear transcription factor-kappaB-dependent cell survival after serotonin 1A receptor activation. *J. Neurochem.* **2005**, *95*, 1653–1666.
23. Adayev, T.; Ray, I.; Sondhi, R.; Sobocki, T.; Banerjee, P. The G protein-coupled 5-HT_{1A} receptor causes suppression of caspase-3 through MAPK and protein kinase Calpha. *Biochim. Biophys. Acta* **2003**, *1640*, 85–96.
24. Polter, A.M.; Li, X. 5-HT_{1A} receptor-regulated signal transduction pathways in brain. *Cell Signal.* **2010**, *22*, 1406–1412.
25. Clarke, W.P.; Yocca, F.D.; Maayani, S. Lack of 5-hydroxytryptamine_{1A}-mediated inhibition of adenylyl cyclase in dorsal raphe of male and female rats. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 1259–1266.
26. Mannoury la, C.C.; El, M.S.; Hanoun, N.; Hamon, M.; Lanfumey, L. Regional differences in the coupling of 5-hydroxytryptamine-1A receptors to G proteins in the rat brain. *Mol. Pharmacol.* **2006**, *70*, 1013–1021.

27. Marazziti, D.; Palego, L.; Giromella, A.; Mazzoni, M.R.; Borsini, F.; Mayer, N.; Naccarato, A.G.; Lucacchini, A.; Cassano, G.B. Region-dependent effects of flibanserin and buspirone on adenylyl cyclase activity in the human brain. *Int. J. Neuropsychopharmacol.* **2002**, *5*, 131–140.
28. Martel, J.C.; Ormiere, A.M.; Leduc, N.; Assie, M.B.; Cussac, D.; Newman-Tancredi, A. Native rat hippocampal 5-HT_{1A} receptors show constitutive activity. *Mol. Pharmacol.* **2007**, *71*, 638–643.
29. Moyano, S.; Del, R.J.; Frechilla, D. Role of hippocampal CaMKII in serotonin 5-HT (1A) receptor-mediated learning deficit in rats. *Neuropsychopharmacology* **2004**, *29*, 2216–2224.
30. Luscher, C.; Jan, L.Y.; Stoffel, M.; Malenka, R.C.; Nicoll, R.A. G protein-coupled inwardly rectifying K⁺ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron* **1997**, *19*, 687–695.
31. Oleskevich, S. G alpha o1 decapeptide modulates the hippocampal 5-HT_{1A} potassium current. *J. Neurophysiol.* **1995**, *74*, 2189–2193.
32. Colino, A.; Halliwell, J.V. Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin. *Nature* **1987**, *328*, 73–77.
33. Andrade, R.; Nicoll, R.A. Pharmacologically distinct actions of serotonin on single pyramidal neurones of the rat hippocampus recorded *in vitro*. *J. Physiol.* **1987**, *394*, 99–124.
34. Clarke, W.P.; De, V.M.; Beck, S.G.; Maayani, S.; Goldfarb, J. Serotonin decreases population spike amplitude in hippocampal cells through a pertussis toxin substrate. *Brain Res.* **1987**, *410*, 357–361.
35. Penington, N.J.; Kelly, J.S.; Fox, A.P. Whole-cell recordings of inwardly rectifying K⁺ currents activated by 5-HT_{1A} receptors on dorsal raphe neurones of the adult rat. *J. Physiol.* **1993**, *469*, 387–405.
36. Bayliss, D.A.; Li, Y.W.; Talley, E.M. Effects of serotonin on caudal raphe neurons: Activation of an inwardly rectifying potassium conductance. *J. Neurophysiol.* **1997**, *77*, 1349–1361.
37. Ehrenguber, M.U.; Doupnik, C.A.; Xu, Y.; Garvey, J.; Jasek, M.C.; Lester, H.A.; Davidson, N. Activation of heteromeric G protein-gated inward rectifier K⁺ channels overexpressed by adenovirus gene transfer inhibits the excitability of hippocampal neurons. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 7070–7075.
38. Loucif, A.J.; Bonnavion, P.; Macri, B.; Golmard, J.L.; Boni, C.; Melfort, M.; Leonard, G.; Lesch, K.P.; Adrien, J.; Jacquin, T.D. Gender-dependent regulation of G-protein-gated inwardly rectifying potassium current in dorsal raphe neurons in knock-out mice devoid of the 5-hydroxytryptamine transporter. *J. Neurobiol.* **2006**, *66*, 1475–1488.
39. Doupnik, C.A.; Davidson, N.; Lester, H.A.; Kofuji, P. RGS proteins reconstitute the rapid gating kinetics of gbetagamma-activated inwardly rectifying K⁺ channels. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 10461–10466.
40. Doupnik, C.A.; Dessauer, C.W.; Slepak, V.Z.; Gilman, A.G.; Davidson, N.; Lester, H.A. Time resolved kinetics of direct G beta 1 gamma 2 interactions with the carboxyl terminus of Kir3.4 inward rectifier K⁺ channel subunits. *Neuropharmacology* **1996**, *35*, 923–931.
41. Kooor, A.; Lester, H.A. Gi Irks GIRKs. *Neuron* **2002**, *33*, 6–8.
42. Tsetsenis, T.; Ma, X.H.; Lo, I.L.; Beck, S.G.; Gross, C. Suppression of conditioning to ambiguous cues by pharmacogenetic inhibition of the dentate gyrus. *Nat. Neurosci.* **2007**, *10*, 896–902.
43. Chen, Y.; Penington, N.J. Differential effects of protein kinase C activation on 5-HT_{1A} receptor coupling to Ca²⁺ and K⁺ currents in rat serotonergic neurones. *J. Physiol.* **1996**, *496*, 129–137.

44. Penington, N.J.; Fox, A.P. Toxin-insensitive Ca current in dorsal raphe neurons. *J. Neurosci.* **1995**, *15*, 5719–5726.
45. Bayliss, D.A.; Li, Y.W.; Talley, E.M. Effects of serotonin on caudal raphe neurons: Inhibition of N- and P/Q-type calcium channels and the afterhyperpolarization. *J. Neurophysiol.* **1997**, *77*, 1362–1374.
46. Chen, Y.; Penington, N.J. QEHA27, a peptide that binds to G-protein beta gamma-subunits, reduces the inhibitory effect of 5-HT on the Ca²⁺ current of rat dorsal raphe neurons. *Neurosci. Lett.* **1997**, *224*, 87–90.
47. Blumer, K.J.; Johnson, G.L. Diversity in function and regulation of MAP kinase pathways. *Trends Biochem. Sci.* **1994**, *19*, 236–240.
48. Garnovskaya, M.N.; van Biesen, T.; Hawe, B.; Ramos, S.C.; Lefkowitz, R.J.; Raymond, J.R. Ras-dependent activation of fibroblast mitogen-activated protein kinase by 5-HT_{1A} receptor via a G protein beta gamma-subunit-initiated pathway. *Biochemistry* **1996**, *35*, 13716–13722.
49. Cowen, D.S.; Sowers, R.S.; Manning, D.R. Activation of a mitogen-activated protein kinase (ERK2) by the 5-hydroxytryptamine_{1A} receptor is sensitive not only to inhibitors of phosphatidylinositol 3-kinase, but to an inhibitor of phosphatidylcholine hydrolysis. *J. Biol. Chem.* **1996**, *271*, 22297–22300.
50. Millan, M.J.; Newman-Tancredi, A.; Duqueyroi, D.; Cussac, D. Agonist properties of pindolol at h5-HT_{1A} receptors coupled to mitogen-activated protein kinase. *Eur. J. Pharmacol.* **2001**, *424*, 13–17.
51. Garnovskaya, M.N.; Mukhin, Y.; Raymond, J.R. Rapid activation of sodium-proton exchange and extracellular signal-regulated protein kinase in fibroblasts by G protein-coupled 5-HT_{1A} receptor involves distinct signalling cascades. *Biochem. J.* **1998**, *330*, 489–495.
52. la Rocca, G.J.; Mukhin, Y.V.; Garnovskaya, M.N.; Daaka, Y.; Clark, G.J.; Luttrell, L.M.; Lefkowitz, R.J.; Raymond, J.R. Serotonin 5-HT_{1A} receptor-mediated Erk activation requires calcium/calmodulin-dependent receptor endocytosis. *J. Biol. Chem.* **1999**, *274*, 4749–4753.
53. Sullivan, N.R.; Crane, J.W.; Damjanoska, K.J.; Carrasco, G.A.; D'Souza, D.N.; Garcia, F.; van de Kar, L.D. Tansospirone activates neuroendocrine and ERK (MAP kinase) signaling pathways specifically through 5-HT_{1A} receptor mechanisms *in vivo*. *Naunyn Schmiedeberg's Arch. Pharmacol.* **2005**, *371*, 18–26.
54. Buritova, J.; Berrichon, G.; Cathala, C.; Colpaert, F.; Cussac, D. Region-specific changes in 5-HT_{1A} agonist-induced Extracellular signal-Regulated Kinases 1/2 phosphorylation in rat brain: A quantitative ELISA study. *Neuropharmacology* **2009**, *56*, 350–361.
55. Newman-Tancredi, A.; Martel, J.C.; Assie, M.B.; Buritova, J.; Lauressergues, E.; Cosi, C.; Heusler, P.; Bruins, S.L.; Colpaert, F.C.; Vacher, B.; *et al.* Signal transduction and functional selectivity of F15599, a preferential post-synaptic 5-HT_{1A} receptor agonist. *Br. J. Pharmacol.* **2009**, *156*, 338–353.
56. Adayev, T.; El-Sherif, Y.; Barua, M.; Penington, N.J.; Banerjee, P. Agonist stimulation of the serotonin_{1A} receptor causes suppression of anoxia-induced apoptosis via mitogen-activated protein kinase in neuronal HN2-5 cells. *J. Neurochem.* **1999**, *72*, 1489–1496.
57. Cowen, D.S.; Johnson-Farley, N.N.; Travkina, T. 5-HT_{1A} receptors couple to activation of Akt, but not extracellular-regulated kinase (ERK), in cultured hippocampal neurons. *J. Neurochem.* **2005**, *93*, 910–917.

58. Druse, M.; Tajuddin, N.F.; Gillespie, R.A.; Le, P. Signaling pathways involved with serotonin1A agonist-mediated neuroprotection against ethanol-induced apoptosis of fetal rhombencephalic neurons. *Brain Res. Dev. Brain Res.* **2005**, *159*, 18–28.
59. Kushwaha, N.; Albert, P.R. Coupling of 5-HT1A autoreceptors to inhibition of mitogen-activated protein kinase activation via G beta gamma subunit signaling. *Eur. J. Neurosci.* **2005**, *21*, 721–732.
60. Crane, J.W.; Shimizu, K.; Carrasco, G.A.; Garcia, F.; Jia, C.; Sullivan, N.R.; D'Souza, D.N.; Zhang, Y.; Van de Kar, L.D.; Muma, N.A.; *et al.* 5-HT1A receptors mediate (+)8-OH-DPAT-stimulation of extracellular signal-regulated kinase (MAP kinase) *in vivo* in rat hypothalamus: Time dependence and regional differences. *Brain Res.* **2007**, *1183*, 51–59.
61. Chen, J.; Shen, C.; Meller, E. 5-HT1A receptor-mediated regulation of mitogen-activated protein kinase phosphorylation in rat brain. *Eur. J. Pharmacol.* **2002**, *452*, 155–162.
62. Sturgill, T.W.; Ray, L.B.; Erikson, E.; Maller, J.L. Insulin-stimulated MAP-2 kinase phosphorylates and activates ribosomal protein S6 kinase II. *Nature* **1988**, *334*, 715–718.
63. Cowen, D.S.; Molinoff, P.B.; Manning, D.R. 5-hydroxytryptamine1A receptor-mediated increases in receptor expression and activation of nuclear factor-kappaB in transfected Chinese hamster ovary cells. *Mol. Pharmacol.* **1997**, *52*, 221–226.
64. Blendy, J.A. The role of CREB in depression and antidepressant treatment. *Biol. Psychiatry* **2006**, *59*, 1144–1150.
65. Einat, H.; Yuan, P.; Gould, T.D.; Li, J.; Du, J.; Zhang, L.; Manji, H.K.; Chen, G. The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. *J. Neurosci.* **2003**, *23*, 7311–7316.
66. Ailing, F.; Fan, L.; Li, S.; Manji, S. Role of extracellular signal-regulated kinase signal transduction pathway in anxiety. *J. Psychiatr. Res.* **2008**, *43*, 55–63.
67. Qi, H.; Mailliet, F.; Spedding, M.; Rocher, C.; Zhang, X.; Delagrangé, P.; McEwen, B.; Jay, T.M.; Svenningsson, P. Antidepressants reverse the attenuation of the neurotrophic MEK/MAPK cascade in frontal cortex by elevated platform stress; reversal of effects on LTP is associated with GluA1 phosphorylation. *Neuropharmacology* **2009**, *56*, 37–46.
68. Creson, T.K.; Hao, Y.; Engel, S.; Shen, Y.; Hamidi, A.; Zhuo, M.; Manji, H.K.; Chen, G. The anterior cingulate ERK pathway contributes to regulation of behavioral excitement and hedonic activity. *Bipolar Disord.* **2009**, *11*, 339–350.
69. Duman, C.H.; Schlesinger, L.; Kodama, M.; Russell, D.S.; Duman, R.S. A role for MAP kinase signaling in behavioral models of depression and antidepressant treatment. *Biol. Psychiatry* **2007**, *61*, 661–670.
70. Huang, W.; Zhao, Y.; Zhu, X.; Cai, Z.; Wang, S.; Yao, S.; Qi, Z.; Xie, P. Fluoxetine upregulates phosphorylated-AKT and phosphorylated-ERK1/2 proteins in neural stem cells: Evidence for a crosstalk between AKT and ERK1/2 pathways. *J. Mol. Neurosci.* **2013**, *49*, 244–249.
71. Sale, E.M.; Sale, G.J. Protein kinase B: Signalling roles and therapeutic targeting. *Cell Mol. Life Sci.* **2008**, *65*, 113–127.
72. Kim, S.J.; Cheon, S.H.; Yoo, S.J.; Kwon, J.; Park, J.H.; Kim, C.G.; Rhee, K.; You, S.; Lee, J.Y.; Roh, S.I.; *et al.* Contribution of the PI3K/Akt/PKB signal pathway to maintenance of self-renewal in human embryonic stem cells. *FEBS Lett.* **2005**, *579*, 534–540.

73. Saunders, C.; Siuta, M.; Robertson, S.D.; Davis, A.R.; Sauer, J.; Matthies, H.J.; Gresch, P.J.; Airey, D.C.; Lindsley, C.W.; Schetz, J.A.; *et al.* Neuronal ablation of p-Akt at Ser473 leads to altered 5-HT_{1A/2A} receptor function. *Neurochem. Int.* **2014**, *73*, 113–121.
74. Liu, Y.; Fanburg, B.L. Serotonin-induced growth of pulmonary artery smooth muscle requires activation of phosphatidylinositol 3-kinase/serine-threonine protein kinase B/mammalian target of rapamycin/p70 ribosomal S6 kinase 1. *Am. J. Respir. Cell Mol. Biol.* **2006**, *34*, 182–191.
75. Cross, D.A.; Alessi, D.R.; Cohen, P.; Andjelkovich, M.; Hemmings, B.A. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* **1995**, *378*, 785–789.
76. Goode, N.; Hughes, K.; Woodgett, J.R.; Parker, P.J. Differential regulation of glycogen synthase kinase-3 beta by protein kinase C isotypes. *J. Biol. Chem.* **1992**, *267*, 16878–16882.
77. Li, M.; Wang, X.; Meintzer, M.K.; Laessig, T.; Birnbaum, M.J.; Heidenreich, K.A. Cyclic AMP promotes neuronal survival by phosphorylation of glycogen synthase kinase 3beta. *Mol. Cell. Biol.* **2000**, *20*, 9356–9363.
78. Klein, P.S.; Melton, D.A. A molecular mechanism for the effect of lithium on development. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 8455–8459.
79. De Sarno, P.; Li, X.; Jope, R.S. Regulation of Akt and glycogen synthase kinase-3 beta phosphorylation by sodium valproate and lithium. *Neuropharmacology* **2002**, *43*, 1158–1164.
80. Kaidanovich-Beilin, O.; Milman, A.; Weizman, A.; Pick, C.G.; Eldar-Finkelman, H. Rapid antidepressive-like activity of specific glycogen synthase kinase-3 inhibitor and its effect on beta-catenin in mouse hippocampus. *Biol. Psychiatry* **2004**, *55*, 781–784.
81. Gould, T.D.; Einat, H.; Bhat, R.; Manji, H.K. AR-A014418, a selective GSK-3 inhibitor, produces antidepressant-like effects in the forced swim test. *Int. J. Neuropsychopharmacol.* **2004**, *7*, 387–390.
82. Beaulieu, J.M.; Sotnikova, T.D.; Yao, W.D.; Kockeritz, L.; Woodgett, J.R.; Gainetdinov, R.R.; Caron, M.G. Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5099–5104.
83. Prickaerts, J.; Moechars, D.; Cryns, K.; Lenaerts, I.; van Craenendonck, H.; Goris, I.; Daneels, G.; Bouwknecht, J.A.; Steckler, T. Transgenic mice overexpressing glycogen synthase kinase 3beta: A putative model of hyperactivity and mania. *J. Neurosci.* **2006**, *26*, 9022–9029.
84. Brunet, A.; Bonni, A.; Zigmond, M.J.; Lin, M.Z.; Juo, P.; Hu, L.S.; Anderson, M.J.; Arden, K.C.; Blenis, J.; Greenberg, M.E. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* **1999**, *96*, 857–868.
85. Liang, B.; Moussaif, M.; Kuan, C.J.; Gargus, J.J.; Sze, J.Y. Serotonin targets the DAF-16/FOXO signaling pathway to modulate stress responses. *Cell Metab.* **2006**, *4*, 429–440.
86. Polter, A.; Yang, S.; Zmijewska, A.A.; van Groen, T.; Paik, J.H.; Depinho, R.A.; Peng, S.L.; Jope, R.S.; Li, X. Forkhead box, class O transcription factors in brain: Regulation and behavioral manifestation. *Biol. Psychiatry* **2009**, *65*, 150–159.
87. Mao, Z.; Liu, L.; Zhang, R.; Li, X. Lithium reduces FoxO3a transcriptional activity by decreasing its intracellular content. *Biol. Psychiatry* **2007**, *62*, 1423–1430.
88. Turner, J.H.; Garnovskaya, M.N.; Coaxum, S.D.; Vlasova, T.M.; Yakutovich, M.; Lefler, D.M.; Raymond, J.R. Ca²⁺-calmodulin and janus kinase 2 are required for activation of sodium-proton exchange by the Gi-coupled 5-hydroxytryptamine 1a receptor. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 314–322.

89. Carmena, M.J.; Camacho, A.; Solano, R.M.; Montalvo, L.; Garcia-Lopez, E.; Arias, A.; Prieto, J.C. 5-hydroxytryptamine_{1A} receptor-mediated effects on adenylate cyclase and nitric oxide synthase activities in rat ventral prostate. *Cell Signal.* **1998**, *10*, 583–587.
90. Shinka, T.; Onodera, D.; Tanaka, T.; Shoji, N.; Miyazaki, T.; Moriuchi, T.; Fukumoto, T. Serotonin synthesis and metabolism-related molecules in a human prostate cancer cell line. *Oncol. Lett.* **2011**, *2*, 211–215.
91. Strosznajder, J.; Chalimoniuk, M.; Samochocki, M. Activation of serotonergic 5-HT_{1A} receptor reduces Ca²⁺- and glutamatergic receptor-evoked arachidonic acid and No/cGMP release in adult hippocampus. *Neurochem. Int.* **1996**, *28*, 439–444.
92. Maura, G.; Marcoli, M.; Pepicelli, O.; Rosu, C.; Viola, C.; Raiteri, M. Serotonin inhibition of the NMDA receptor/nitric oxide/cyclic GMP pathway in human neocortex slices: Involvement of 5-HT_{2C} and 5-HT_{1A} receptors. *Br. J. Pharmacol.* **2000**, *130*, 1853–1858.
93. Volke, V.; Wegener, G.; Bourin, M.; Vasar, E. Antidepressant- and anxiolytic-like effects of selective neuronal NOS inhibitor 1-(2-trifluoromethylphenyl)-imidazole in mice. *Behav. Brain Res.* **2003**, *140*, 141–147.
94. Yildiz, F.; Erden, B.F.; Ulak, G.; Utkan, T.; Gacar, N. Antidepressant-like effect of 7-nitroindazole in the forced swimming test in rats. *Psychopharmacology* **2000**, *149*, 41–44.
95. Ulak, G.; Mutlu, O.; Tanyeri, P.; Komsuoglu, F.I.; Akar, F.Y.; Erden, B.F. Involvement of serotonin receptor subtypes in the antidepressant-like effect of TRIM in the rat forced swimming test. *Pharmacol. Biochem. Behav.* **2010**, *95*, 308–314.
96. Heusler, P.; Pauwels, P.J.; Wurch, T.; Newman-Tancredi, A.; Tytgat, J.; Colpaert, F.C.; Cussac, D. Differential ion current activation by human 5-HT_{1A} receptors in *Xenopus* oocytes: Evidence for agonist-directed trafficking of receptor signalling. *Neuropharmacology* **2005**, *49*, 963–976.
97. Shopsin, B.; Friedman, E.; Gershon, S. Parachlorophenylalanine reversal of tranylcypromine effects in depressed patients. *Arch. Gen. Psychiatry* **1976**, *33*, 811–819.
98. Young, S.N.; Smith, S.E.; Pihl, R.O.; Ervin, F.R. Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology* **1985**, *87*, 173–177.
99. Stark, P.; Hardison, C.D. A review of multicenter controlled studies of fluoxetine vs. imipramine and placebo in outpatients with major depressive disorder. *J. Clin. Psychiatry* **1985**, *46*, 53–58.
100. Franklin, M.; Bermudez, I.; Murck, H.; Singewald, N.; Gaburro, S. Sub-chronic dietary tryptophan depletion--an animal model of depression with improved face and good construct validity. *J. Psychiatr. Res.* **2012**, *46*, 239–247.
101. Chau, D.T.; Rada, P.V.; Kim, K.; Kosloff, R.A.; Hoebel, B.G. Fluoxetine alleviates behavioral depression while decreasing acetylcholine release in the nucleus accumbens shell. *Neuropsychopharmacology* **2011**, *36*, 1729–1737.
102. Baudry, A.; Mouillet-Richard, S.; Schneider, B.; Launay, J.M.; Kellermann, O. miR-16 targets the serotonin transporter: A new facet for adaptive responses to antidepressants. *Science* **2010**, *329*, 1537–1541.
103. Temel, Y.; Boothman, L.J.; Blokland, A.; Magill, P.J.; Steinbusch, H.W.; Visser-Vandewalle, V.; Sharp, T. Inhibition of 5-HT neuron activity and induction of depressive-like behavior by high-frequency stimulation of the subthalamic nucleus. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 17087–17092.

104. Gross, C.; Santarelli, L.; Brunner, D.; Zhuang, X.; Hen, R. Altered fear circuits in 5-HT (1A) receptor KO mice. *Biol. Psychiatry* **2000**, *48*, 1157–1163.
105. Kusserow, H.; Davies, B.; Hortnagl, H.; Voigt, I.; Stroh, T.; Bert, B.; Deng, D.R.; Fink, H.; Veh, R.W.; Theuring, F. Reduced anxiety-related behaviour in transgenic mice overexpressing serotonin 1A receptors. *Brain Res. Mol. Brain Res.* **2004**, *129*, 104–116.
106. Heisler, L.K.; Chu, H.M.; Brennan, T.J.; Danao, J.A.; Bajwa, P.; Parsons, L.H.; Tecott, L.H. Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 15049–15054.
107. Klemenhagen, K.C.; Gordon, J.A.; David, D.J.; Hen, R.; Gross, C.T. Increased fear response to contextual cues in mice lacking the 5-HT1A receptor. *Neuropsychopharmacology* **2006**, *31*, 101–111.
108. Parks, C.L.; Robinson, P.S.; Sibille, E.; Shenk, T.; Toth, M. Increased anxiety of mice lacking the serotonin1A receptor. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 10734–10739.
109. Ramboz, S.; Oosting, R.; Amara, D.A.; Kung, H.F.; Blier, P.; Mendelsohn, M.; Mann, J.J.; Brunner, D.; Hen, R. Serotonin receptor 1A knockout: An animal model of anxiety-related disorder. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 14476–14481.
110. Gunther, L.; Rothe, J.; Rex, A.; Voigt, J.P.; Millan, M.J.; Fink, H.; Bert, B. 5-HT(1A)-receptor over-expressing mice: Genotype and sex dependent responses to antidepressants in the forced swim-test. *Neuropharmacology* **2011**, *61*, 433–441.
111. Maletic, V.; Robinson, M.; Oakes, T.; Iyengar, S.; Ball, S.G.; Russell, J. Neurobiology of depression: An integrated view of key findings. *Int. J. Clin. Pract.* **2007**, *61*, 2030–2040.
112. Richardson-Jones, J.W.; Craige, C.P.; Nguyen, T.H.; Kung, H.F.; Gardier, A.M.; Dranovsky, A.; David, D.J.; Guiard, B.P.; Beck, S.G.; Hen, R.; *et al.* Serotonin-1A autoreceptors are necessary and sufficient for the normal formation of circuits underlying innate anxiety. *J. Neurosci.* **2011**, *31*, 6008–6018.
113. Donaldson, Z.R.; Piel, D.A.; Santos, T.L.; Richardson-Jones, J.; Leonardo, E.D.; Beck, S.G.; Champagne, F.A.; Hen, R. Developmental effects of serotonin 1A autoreceptors on anxiety and social behavior. *Neuropsychopharmacology* **2014**, *39*, 291–302.
114. Bortolozzi, A.; Castane, A.; Semakova, J.; Santana, N.; Alvarado, G.; Cortes, R.; Ferres-Coy, A.; Fernandez, G.; Carmona, M.C.; Toth, M.; *et al.* New antidepressant strategy based on acute siRNA silencing of 5-HT1A autoreceptors. *Mol. Psychiatry* **2012**, *17*, 567.
115. Albert, P.R.; Francois, B.L. Modifying 5-HT1A receptor gene expression as a new target for antidepressant therapy. *Front. Neurosci.* **2010**, *4*, 35.
116. Ferres-Coy, A.; Santana, N.; Castane, A.; Cortes, R.; Carmona, M.C.; Toth, M.; Montefeltro, A.; Artigas, F.; Bortolozzi, A. Acute 5-HT(1A) autoreceptor knockdown increases antidepressant responses and serotonin release in stressful conditions. *Psychopharmacology* **2013**, *225*, 61–74.
117. Bortolozzi, A.; Castane, A.; Semakova, J.; Santana, N.; Alvarado, G.; Cortes, R.; Ferres-Coy, A.; Fernandez, G.; Carmona, M.C.; Toth, M.; *et al.* Selective siRNA-mediated suppression of 5-HT1A autoreceptors evokes strong anti-depressant-like effects. *Mol. Psychiatry* **2012**, *17*, 612–623.
118. Bordukalo-Niksic, T.; Mokrovic, G.; Stefulj, J.; Zivin, M.; Jernej, B.; Cicin-Sain, L. 5HT-1A receptors and anxiety-like behaviours: Studies in rats with constitutionally upregulated/downregulated serotonin transporter. *Behav. Brain Res.* **2010**, *213*, 238–245.

119. Assie, M.B.; Lomenech, H.; Ravailhe, V.; Faucillon, V.; Newman-Tancredi, A. Rapid desensitization of somatodendritic 5-HT_{1A} receptors by chronic administration of the high-efficacy 5-HT_{1A} agonist, F13714: A microdialysis study in the rat. *Br. J. Pharmacol.* **2006**, *149*, 170–178.
120. Depoortere, R.; Auclair, A.L.; Bardin, L.; Colpaert, F.C.; Vacher, B.; Newman-Tancredi, A. F15599, a preferential post-synaptic 5-HT_{1A} receptor agonist: Activity in models of cognition in comparison with reference 5-HT_{1A} receptor agonists. *Eur. Neuropsychopharmacol.* **2010**, *20*, 641–654.
121. Llado-Pelfort, L.; Assie, M.B.; Newman-Tancredi, A.; Artigas, F.; Celada, P. Preferential *in vivo* action of F15599, a novel 5-HT(1A) receptor agonist, at postsynaptic 5-HT(1A) receptors. *Br. J. Pharmacol.* **2010**, *160*, 1929–1940.
122. Assie, M.B.; Bardin, L.; Auclair, A.L.; Carilla-Durand, E.; Depoortere, R.; Koek, W.; Kleven, M.S.; Colpaert, F.; Vacher, B.; Newman-Tancredi, A. F15599, a highly selective post-synaptic 5-HT(1A) receptor agonist: *In vivo* profile in behavioural models of antidepressant and serotonergic activity. *Int. J. Neuropsychopharmacol.* **2010**, *13*, 1285–1298.
123. Savitz, J.; Lucki, I.; Drevets, W.C. 5-HT (1A) receptor function in major depressive disorder. *Prog. Neurobiol.* **2009**, *88*, 17–31.
124. Lemonde, S.; Turecki, G.; Bakish, D.; Du, L.; Hrdina, P.D.; Bown, C.D.; Sequeira, A.; Kushwaha, N.; Morris, S.J.; Basak, A.; *et al.* Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. *J. Neurosci.* **2003**, *23*, 8788–8799.
125. Stockmeier, C.A.; Shapiro, L.A.; Dilley, G.E.; Kolli, T.N.; Friedman, L.; Rajkowska, G. Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression-postmortem evidence for decreased serotonin activity. *J. Neurosci.* **1998**, *18*, 7394–7401.
126. Neff, C.D.; Abkevich, V.; Packer, J.C.; Chen, Y.; Potter, J.; Riley, R.; Davenport, C.; DeGrado, W.J.; Jammulapati, S.; Bhatena, A.; *et al.* Evidence for HTR1A and LHPP as interacting genetic risk factors in major depression. *Mol. Psychiatry* **2009**, *14*, 621–630.
127. Sargent, P.A.; Kjaer, K.H.; Bench, C.J.; Rabiner, E.A.; Messa, C.; Meyer, J.; Gunn, R.N.; Grasby, P.M.; Cowen, P.J. Brain serotonin_{1A} receptor binding measured by positron emission tomography with [¹¹C]WAY-100635: Effects of depression and antidepressant treatment. *Arch. Gen. Psychiatry* **2000**, *57*, 174–180.
128. Bhagwagar, Z.; Rabiner, E.A.; Sargent, P.A.; Grasby, P.M.; Cowen, P.J. Persistent reduction in brain serotonin_{1A} receptor binding in recovered depressed men measured by positron emission tomography with [¹¹C]WAY-100635. *Mol. Psychiatry* **2004**, *9*, 386–392.
129. Artigas, F.; Romero, L.; de, M.C.; Blier, P. Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT_{1A} antagonists. *Trends Neurosci.* **1996**, *19*, 378–383.
130. Nord, M.; Finnema, S.J.; Halldin, C.; Farde, L. Effect of a single dose of escitalopram on serotonin concentration in the non-human and human primate brain. *Int. J. Neuropsychopharmacol.* **2013**, *16*, 1577–1586.
131. Artigas, F.; Perez, V.; Alvarez, E. Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. *Arch. Gen. Psychiatry* **1994**, *51*, 248–251.

132. Portella, M.J.; de Diego-Adelino, J.; Ballesteros, J.; Puigdemont, D.; Oller, S.; Santos, B.; Alvarez, E.; Artigas, F.; Perez, V. Can we really accelerate and enhance the selective serotonin reuptake inhibitor antidepressant effect? A randomized clinical trial and a meta-analysis of pindolol in nonresistant depression. *J. Clin. Psychiatry* **2011**, *72*, 962–969.
133. Whale, R.; Terao, T.; Cowen, P.; Freemantle, N.; Geddes, J. Pindolol augmentation of serotonin reuptake inhibitors for the treatment of depressive disorder: A systematic review. *J. Psychopharmacol.* **2010**, *24*, 513–520.
134. Bel, N.; Artigas, F. Chronic treatment with fluvoxamine increases extracellular serotonin in frontal cortex but not in raphe nuclei. *Synapse* **1993**, *15*, 243–245.
135. Blier, P.; Pineyro, G.; el Mansari, M.M.; Bergeron, R.; de, M.C. Role of somatodendritic 5-HT autoreceptors in modulating 5-HT neurotransmission. *Ann. N. Y. Acad. Sci.* **1998**, *861*, 204–216.
136. Blier, P.; Ward, N.M. Is there a role for 5-HT_{1A} agonists in the treatment of depression? *Biol. Psychiatry* **2003**, *53*, 193–203.
137. Blier, P.; de, M.C. Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} agonist gepirone: Electrophysiological studies in the rat brain. *Synapse* **1987**, *1*, 470–480.
138. Wieland, S.; Lucki, I. Antidepressant-like activity of 5-HT_{1A} agonists measured with the forced swim test. *Psychopharmacology* **1990**, *101*, 497–504.
139. Haddjeri, N.; Blier, P.; de, M.C. Long-term antidepressant treatments result in a tonic activation of forebrain 5-HT_{1A} receptors. *J. Neurosci.* **1998**, *18*, 10150–10156.
140. Scorza, M.C.; Llado-Pelfort, L.; Oller, S.; Cortes, R.; Puigdemont, D.; Portella, M.J.; Perez-Egea, R.; Alvarez, E.; Celada, P.; Perez, V.; *et al.* Preclinical and clinical characterization of the selective 5-HT_{1A} receptor antagonist DU-125530 for antidepressant treatment. *Br. J. Pharmacol.* **2012**, *167*, 1021–1034.
141. Stahl, S.M.; Kaiser, L.; Roeschen, J.; Keppel Hesselink, J.M.; Orazem, J. Effectiveness of ipsapirone, a 5-HT_{1A} partial agonist, in major depressive disorder: Support for the role of 5-HT_{1A} receptors in the mechanism of action of serotonergic antidepressants. *Int. J. Neuropsychopharmacol.* **1998**, *1*, 11–18.
142. Sprouse, J.S.; Aghajanian, G.K. Responses of hippocampal pyramidal cells to putative serotonin 5-HT_{1A} and 5-HT_{1B} agonists: A comparative study with dorsal raphe neurons. *Neuropharmacology* **1988**, *27*, 707–715.
143. Lemoine, L.; Verduran, M.; Vacher, B.; Blanc, E.; Le Bars, D.; Newman-Tancredi, A.; Zimmer, L. [¹⁸F]F15599, a novel 5-HT_{1A} receptor agonist, as a radioligand for PET neuroimaging. *Eur. J. Nucl. Med. Mol. Imaging* **2010**, *37*, 594–605.
144. Artigas, F. 5-HT and antidepressants: New views from microdialysis studies. *Trends Pharmacol. Sci.* **1993**, *14*, 262.
145. Nestler, E.J.; Barrot, M.; DiLeone, R.J.; Eisch, A.J.; Gold, S.J.; Monteggia, L.M. Neurobiology of depression. *Neuron* **2002**, *34*, 13–25.
146. Duman, R.S.; Monteggia, L.M. A neurotrophic model for stress-related mood disorders. *Biol. Psychiatry* **2006**, *59*, 1116–1127.

147. Page, M.E.; Cryan, J.F.; Sullivan, A.; Dalvi, A.; Saucy, B.; Manning, D.R.; Lucki, I. Behavioral and neurochemical effects of 5-(4-[4-(5-Cyano-3-indolyl)-butyl]-butyl)-1-piperazinyl-benzofuran-2-carboxamide (EMD 68843): A combined selective inhibitor of serotonin reuptake and 5-hydroxytryptamine(1A) receptor partial agonist. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 1220–1227.
148. Mork, A.; Pehrson, A.; Brennum, L.T.; Nielsen, S.M.; Zhong, H.; Lassen, A.B.; Miller, S.; Westrich, L.; Boyle, N.J.; Sanchez, C.; *et al.* Pharmacological effects of Lu AA21004: A novel multimodal compound for the treatment of major depressive disorder. *J. Pharmacol. Exp. Ther.* **2012**, *340*, 666–675.
149. Mayorga, A.J.; Dalvi, A.; Page, M.E.; Zimov-Levinson, S.; Hen, R.; Lucki, I. Antidepressant-like behavioral effects in 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B) receptor mutant mice. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 1101–1107.
150. Duman, R.S. Depression: A case of neuronal life and death? *Biol. Psychiatry* **2004**, *56*, 140–145.
151. Lucas, G.; Rymar, V.V.; Du, J.; Mnie-Filali, O.; Bisgaard, C.; Manta, S.; Lambas-Senas, L.; Wiborg, O.; Haddjeri, N.; Pineyro, G.; *et al.* Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. *Neuron* **2007**, *55*, 712–725.
152. Schaffer, D.V.; Gage, F.H. Neurogenesis and neuroadaptation. *Neuromol. Med.* **2004**, *5*, 1–9.
153. Santarelli, L.; Saxe, M.; Gross, C.; Surget, A.; Battaglia, F.; Dulawa, S.; Weisstaub, N.; Lee, J.; Duman, R.; Arancio, O.; *et al.* Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **2003**, *301*, 805–809.
154. Jacobs, B.L.; van Praag, H.; Gage, F.H. Adult brain neurogenesis and psychiatry: A novel theory of depression. *Mol. Psychiatry* **2000**, *5*, 262–269.
155. Taylor, C.; Fricker, A.D.; Devi, L.A.; Gomes, I. Mechanisms of action of antidepressants: From neurotransmitter systems to signaling pathways. *Cell Signal.* **2005**, *17*, 549–557.
156. Blier, P.; de, M.C. Current advances and trends in the treatment of depression. *Trends Pharmacol. Sci.* **1994**, *15*, 220–226.
157. Neumaier, J.F.; Root, D.C.; Hamblin, M.W. Chronic fluoxetine reduces serotonin transporter mRNA and 5-HT1B mRNA in a sequential manner in the rat dorsal raphe nucleus. *Neuropsychopharmacology* **1996**, *15*, 515–522.
158. Gobert, A.; Rivet, J.M.; Cistarelli, L.; Millan, M.J. Potentiation of the fluoxetine-induced increase in dialysate levels of serotonin (5-HT) in the frontal cortex of freely moving rats by combined blockade of 5-HT1A and 5-HT1B receptors with WAY 100,635 and GR 127,935. *J. Neurochem.* **1997**, *68*, 1159–1163.
159. Gardier, A.M.; Trillat, A.C.; Malagie, I.; David, D.; Hascoet, M.; Colombel, M.C.; Jolliet, P.; Jacquot, C.; Hen, R.; Bourin, M. 5-HT1B serotonin receptors and antidepressant effects of selective serotonin reuptake inhibitors. *C. R. Acad. Sci. III* **2001**, *324*, 433–441.
160. Artigas, F.; Celada, P.; Laruelle, M.; Adell, A. How does pindolol improve antidepressant action? *Trends Pharmacol. Sci.* **2001**, *22*, 224–228.
161. Albert, P.R.; Vahid-Ansari, F.; Luckhart, C. Serotonin-prefrontal cortical circuitry in anxiety and depression phenotypes: Pivotal role of pre- and post-synaptic 5-HT1A receptor expression. *Front. Behav. Neurosci.* **2014**, *8*, 199.
162. De Almeida, J.; Mengod, G. Serotonin 1A receptors in human and monkey prefrontal cortex are mainly expressed in pyramidal neurons and in a GABAergic interneuron subpopulation: Implications for schizophrenia and its treatment. *J. Neurochem.* **2008**, *107*, 488–496.

163. Santana, N.; Bortolozzi, A.; Serrats, J.; Mengod, G.; Artigas, F. Expression of serotonin1A and serotonin2A receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cereb. Cortex* **2004**, *14*, 1100–1109.
164. Amargos-Bosch, M.; Bortolozzi, A.; Puig, M.V.; Serrats, J.; Adell, A.; Celada, P.; Toth, M.; Mengod, G.; Artigas, F. Co-expression and *in vivo* interaction of serotonin1A and serotonin2A receptors in pyramidal neurons of prefrontal cortex. *Cereb. Cortex* **2004**, *14*, 281–299.
165. Bevilacqua, L.; Doly, S.; Kaprio, J.; Yuan, Q.; Tikkanen, R.; Paunio, T.; Zhou, Z.; Wedenoja, J.; Maroteaux, L.; Diaz, S.; *et al.* A population-specific HTR2B stop codon predisposes to severe impulsivity. *Nature* **2010**, *468*, 1061–1066.
166. Launay, J.M.; Schneider, B.; Loric, S.; Da, P.M.; Kellermann, O. Serotonin transport and serotonin transporter-mediated antidepressant recognition are controlled by 5-HT2B receptor signaling in serotonergic neuronal cells. *FASEB J.* **2006**, *20*, 1843–1854.
167. Doly, S.; Bertran-Gonzalez, J.; Callebert, J.; Bruneau, A.; Banas, S.M.; Belmer, A.; Boutourlinsky, K.; Herve, D.; Launay, J.M.; Maroteaux, L. Role of serotonin via 5-HT2B receptors in the reinforcing effects of MDMA in mice. *PLoS ONE* **2009**, *4*, e7952.
168. Doly, S.; Valjent, E.; Setola, V.; Callebert, J.; Herve, D.; Launay, J.M.; Maroteaux, L. Serotonin 5-HT2B receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release *in vivo* and *in vitro*. *J. Neurosci.* **2008**, *28*, 2933–2940.
169. Banas, S.M.; Doly, S.; Boutourlinsky, K.; Diaz, S.L.; Belmer, A.; Callebert, J.; Collet, C.; Launay, J.M.; Maroteaux, L. Deconstructing antiobesity compound action: Requirement of serotonin 5-HT2B receptors for dexfenfluramine anorectic effects. *Neuropsychopharmacology* **2011**, *36*, 423–433.
170. Diaz, S.L.; Maroteaux, L. Implication of 5-HT (2B) receptors in the serotonin syndrome. *Neuropharmacology* **2011**, *61*, 4954502.
171. Diaz, S.L.; Doly, S.; Narboux-Neme, N.; Fernandez, S.; Mazot, P.; Banas, S.M.; Boutourlinsky, K.; Moutkine, I.; Belmer, A.; Roumier, A.; *et al.* 5-HT(2B) receptors are required for serotonin-selective antidepressant actions. *Mol. Psychiatry* **2012**, *17*, 154–163.
172. Rosenzweig-Lipson, S.; Sabb, A.; Stack, G.; Mitchell, P.; Lucki, I.; Malberg, J.E.; Grauer, S.; Brennan, J.; Cryan, J.F.; Sukoff Rizzo, S.J.; *et al.* Antidepressant-like effects of the novel, selective, 5-HT2C receptor agonist WAY-163909 in rodents. *Psychopharmacology (Berl.)* **2007**, *192*, 159–170.
173. Cryan, J.F.; Lucki, I. Antidepressant-like behavioral effects mediated by 5-Hydroxytryptamine (2C) receptors. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 1120–1126.
174. Hervas, I.; Vilario, M.T.; Romero, L.; Scorza, M.C.; Mengod, G.; Artigas, F. Desensitization of 5-HT(1A) autoreceptors by a low chronic fluoxetine dose effect of the concurrent administration of WAY-100635. *Neuropsychopharmacology* **2001**, *24*, 11–20.
175. Riad, M.; Zimmer, L.; Rbahi, L.; Watkins, K.C.; Hamon, M.; Descarries, L. Acute treatment with the antidepressant fluoxetine internalizes 5-HT1A autoreceptors and reduces the *in vivo* binding of the PET radioligand [18F]MPPF in the nucleus raphe dorsalis of rat. *J. Neurosci.* **2004**, *24*, 5420–5426.